Division of Agricultural Sciences
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COMMERCIAL MASS CULTURE of the CALIFORNIA RED SCALE PARASITE

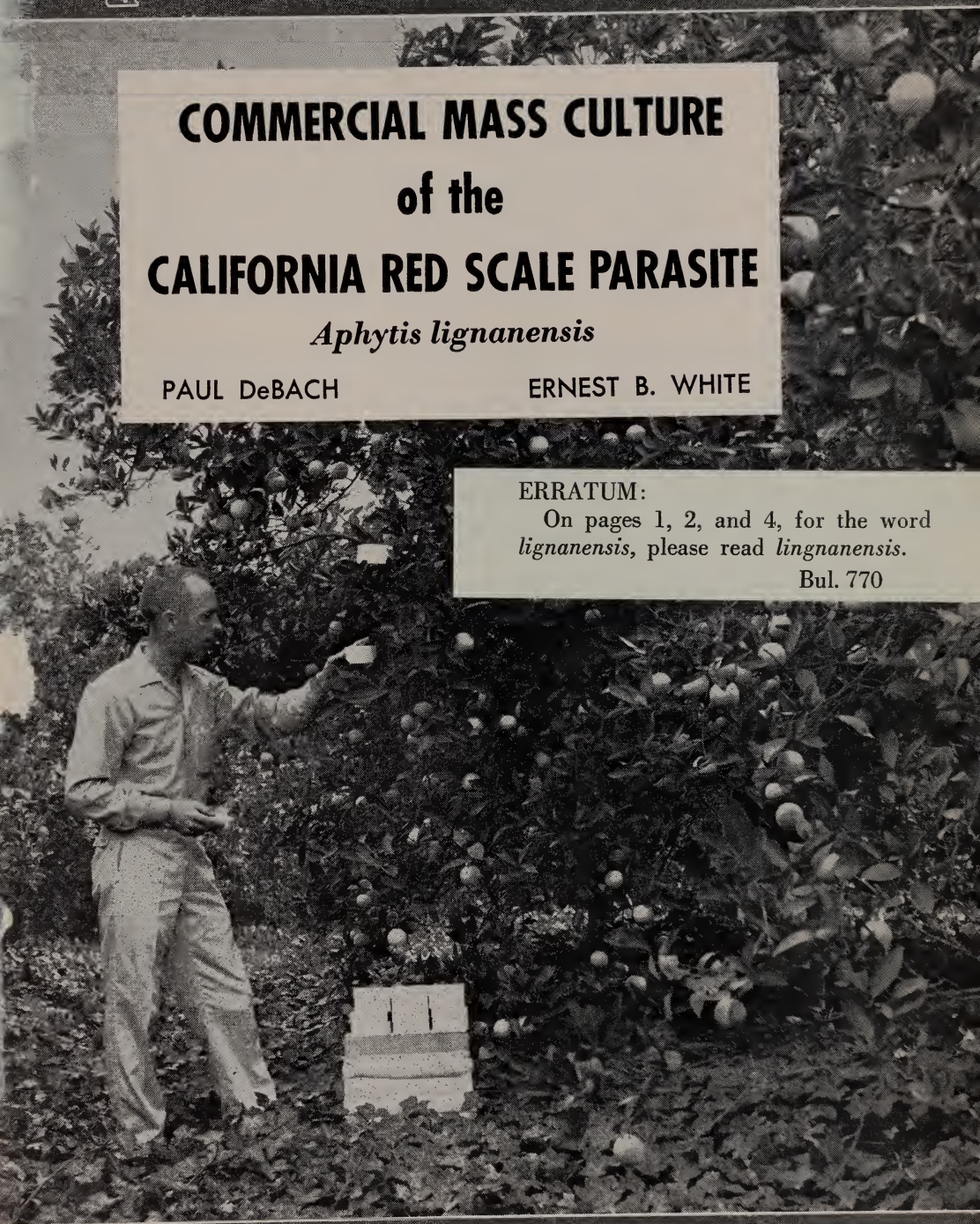
Aphytis lignanensis

PAUL DeBACH ERNEST B. WHITE

ERRATUM:

On pages 1, 2, and 4, for the word *lignanensis*, please read *lingnanensis*.

Bul. 770



CALIFORNIA AGRICULTURAL EXPERIMENT STATION

BULLETIN 770

New techniques for rearing and releasing red-scale parasites are described in this bulletin. These may make it possible to control the scale as cheaply by parasites as by sprays. The method should prove practical for interior citrus areas as soon as strains of the parasite adapted to those areas are developed. Such strains are now being bred.

Aphytis lignanensis **Comp.,** imported from China, is an effective parasite of the California red scale, *Aonidiella aurantii* Mask., in some areas of southern California.

In other areas its efficiency is reduced, principally because of less favorable climatic conditions. But monthly colonizations during a 9-month period, at the rate of 4,000 female parasites per tree per year, give adequate scale control in the intermediate climatic areas of southern California. High- and low-temperature-resistant strains, now being developed, promise comparable results in the interior areas.

Such colonizations would be practical only if they cost no more than the insecticide treatments they would replace—about $40 per acre per year. With previous mass-culture methods costs would have been far higher than this. This bulletin reports the results of attempts to devise more economical methods.

With the method described here, used in an insectary serving a minimum of 400 acres, the cost of colonization is conservatively estimated at $40 per acre or less.

The method (summarized on pages 25–26) is based upon the use of the oleander scale (uniparental strain) as the laboratory host scale and banana squash as the host plant.

Innovations in insectary techniques that are important for economical operation are:

- Newly emerged female parasites are allowed to oviposit for one day in the insectary; then are collected and colonized in the field. The one-day oviposition period provides enough parasites to maintain the insectary population in equilibrium; and the elimination of a separate "mother" culture of parasites simplifies insectary operations.

- Newly producing scale are used 2 weeks for crawler production to maintain the insectary scale population; then are used for parasite production. This eliminates a separate "mother stock" for scale.

- Crawlers are automatically collected from producing scale by a "shadow-line" technique, which also helps separate them from any unwanted parasites or predators that may be present.

- The parasites "sting" the scale in compartments that give improved parasitization and isolation from possible contaminating pests.

- Parasites are anesthetized and collected mechanically, then counted volumetrically for field packaging or use in "stinging" scale.

Efficient and economical operation also demands dense scale infestation of the squash, proper adjustment of the number of *Aphytis* used for stinging to scale density, close control of temperatures and humidities in different parts of the insectary, efficient arrangement of equipment, careful isolation of scale-producing and parasite-producing operations, and effective control of insectary pests.

The experiments and theories upon which the method is based are reported in the first part of the bulletin. A detailed description of recommended techniques begins on page 25.

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INTRODUCTION

Full-time field investigations designed to evaluate the relative importance of various biotic factors influencing populations of the California red scale, *Aonidiella aurantii* (Mask.), on citrus were begun in January, 1948. In order to eliminate from consideration any effect of insecticides on relations between host and natural enemy, citrus groves were sought out which had not been treated with any insecticides whatsoever for from several to many years. Certain of these groves had exceptionally light infestations of California red scale. By the summer of 1948 it was evident that a parasite, the golden chalcid, *Aphytis chrysomphali* (Mercet), was almost solely responsible for control in the groves having the very light scale infestations.

The question naturally arose as to why such control of the red scale by *Aphytis chrysomphali* was not more general. The answer logically seemed to lie in possible adverse effects on the parasite produced by (1) insecticidal treatments and (2) an unfavorable physical environment. It was thought possible that these two influences on the efficiency of the parasite might be alleviated by artificial colonization of large numbers of parasites in the field. Where natural physical environmental (abiotic) factors were periodically unfavorable to *A. chrysomphali*, it was thought that periodic colonization of the parasites at regular intervals might be necessary to establish and maintain a suitable balance; and where the environment was favorable to *A. chrysomphali* but regular insecticidal treatments against the red scale or other pests prevented the establishment of a natural balance, then perhaps an initial colonization of parasites might serve to bring about satisfactory biological control if the interfering treatment could be suspended or modified. As a consequence, mass production of *A. chrysomphali* for use in tests on the efficacy of periodic colonization was planned for 1949.

Meanwhile a different and then undescribed parasite species, *Aphytis lingnanensis*[3] Comp., had been imported from China and colonized for the first time on California red scale in July of 1948. By fall, *A. lingnanensis* was reproducing freely in the field. The efficiency of this parasite appeared to be at least as great as that of the naturally occurring *A. chrysomphali*, and, inasmuch as the new species demonstrated

[1] Submitted for publication March 31, 1958.
[2] Paper No. 1153, University of California Citrus Experiment Station, Riverside, California.
[3] Formerly designated *Aphytis "A."*

certain biological and morphological differences from *chrysomphali*, plans were immediately adopted to rear it in large numbers for field testing in conjunction with *chrysomphali* in 1949.

As these studies continued during the next few years the answer to the question propounded above became clear. According to DeBach, Fisher, and Landi (1955, p. 751):

> All the field evidence in the present case indicates that the various components of climate are the major factors keeping *Aphytis lingnanensis* and *A. chrysomphali* from successfully controlling the California red scale in certain citrus areas. For instance, in the mild coastal climatic zone of southern California natural control of California red scale (control achieved without artificial periodic colonization of parasites) by *A. chrysomphali* or *A. lingnanensis* has occurred in every untreated citrus grove studied. In the less mild intermediate climatic zone, natural control of California red scale by *Aphytis* has occurred in certain groves and not in others, depending apparently upon microclimatic differences. In this intermediate zone, though, it has been possible to obtain biological control of the California red scale by yearly periodic colonizations of large numbers of *Aphytis* in all plots thus far studied. In the interior valley climatic zone, where extremes are greater, natural control rarely if ever occurs, and it has not been possible to achieve a consistent degree of successful biological control through the periodic colonization of parasites.

Aphytis lingnanensis proved to be so much easier to propagate in the insectary than *A. chrysomphali* that more of the former than of the latter were used in periodic colonization tests in 1949. Colonization of equal numbers of each species in comparative plots within the same groves during 1949 showed that *A. lingnanensis* possessed a decided superiority over *A. chrysomphali* under equal competitive conditions. Consequently, from 1950 on, the development of mass-culture techniques was restricted to *A. lingnanensis*.

B. R. Bartlett was in charge of the mass-culture work during 1949 (Bartlett and Fisher, 1950)[4] and S. E. Flanders during part of 1950 (Flanders, 1951). The senior author, who had commenced field investigations of the problem in January, 1948, assumed charge of this phase of the work in the summer of 1950.

As a result of the preliminary data obtained in a limited number of groves in 1949 it appeared that periodic colonization of a total of 100,000 *Aphytis lingnanensis* females per acre of citrus per year might be sufficient (DeBach, Dietrick *et al.*, 1950). Results from field plots in 1950 caused an upward revision of this figure to 400,000, to be colonized in equal numbers each month from March through November.

The philosophy behind this periodic colonization was not to overwhelm the red scale by "inundative" means, comparable to spraying, as suggested by Flanders, but to colonize parasites periodically in sufficient numbers so that after any sort of period adverse to parasite populations, sufficient new stock would be present in the field to build up rapidly and control potential increase of scale population. In no sense was this comparable to spraying, because adequate reproduction of the colonized parasites is necessary. The colonization program of 400,000 female parasites per acre per year means about 4,000 female parasites per tree per year. It becomes obvious that satisfactory reproduction of the colonized parasites and their progeny is necessary when it is realized that on a heavily scale-infested citrus tree a single fruit may bear 2,000 to 4,000 red scales and the entire tree from one to several million red scales.

Thus, the goal which became defined in 1950 involved the production and colonization of 400,000 female *Aphytis lingnanensis* per acre per year at a cost equal to or less than that required for insecticidal control of red scale (esti-

[4] See "Literature Cited" at the end of the bulletin for citations, which are referred to in the text by author and date of publication.

mated to be about $40 per acre) and on an efficient enough basis so that a small insectary could service large acreages. Monthly parasite production would need to be virtually constant to meet these requirements.

Although usually sufficient for the needs of experimental tests of periodic colonization, the methods developed by Bartlett and Fisher and by Flanders were inadequate to meet the goal set forth above. Neither method was sufficiently economical or efficient. Some major reasons for this were: too much insectary space was required; too much labor was involved; host plants for rearing the scales were unsatisfactory for various reasons; and per cent parasitization was poor.

The current method did not evolve from the earlier ones but represents a nearly complete change in techniques dictated by experimental comparisons between previous techniques and new alternatives. The reason why changes were necessary and why a certain new technique was adopted are set down in the sections that follow.

In order to maintain sufficient parasite production for rather extensive field-colonization tests and at the same time try to develop a more efficient mass-culture method, more insectary space and personnel were found to be necessary than were available at Riverside in 1950. Consequently, in view of the need and the interest of citrus industry leaders in the project, a special budget was granted by the Regents of the University of California to expedite the work. The need for additional insectary space was met by the generous offer of the use of the Los Angeles County Insectary at Rivera by Mr. H. J. Ryan, Los Angeles County Commissioner of Agriculture.

The method described in this paper was developed largely at the Los Angeles County Insectary under the direct supervision of the junior author.

Results of Field Colonizations and Future Possibilities. Results of periodic field colonization of *Aphytis lingnanensis* have been discussed by DeBach, Landi, and White (1955). Conclusions were as follows:

> Using such a program good results were obtained in nearly all test plots in intermediate [climatic] areas. Indications were that fewer numbers of parasites [than 400,000 per acre] would be sufficient in some groves or in certain years. In intermediate areas a commercial insectary program of parasite production for red scale control would appear feasible.
>
> However, in interior citrus areas the results for the most part were unsatisfactory. Certain plots, having a favorable microclimate, may be maintained under biological control. Such a plot in a warm winter location at the Citrus Experiment Station has been kept under satisfactory control by this program since 1949.
>
> On the average, however, good commercial control could not be guaranteed in interior citrus areas.

It now appears unlikely that citrus growers in intermediate climatic areas of southern California will undertake a commercial insectary program for production of parasites for red scale control because so much of this area is being subdivided for home construction that the group organization necessary for such a project is disrupted.

This does not, however, preclude the possibility of rather widespread application of the periodic colonization method. When it became apparent that the parasites usually did not effect biological control in the interior citrus areas because climatic extremes killed too many parasites, we began a program for the development of temperature-tolerant strains of *Aphytis lingnanensis* with the idea of developing a cold-tolerant strain which could be colonized in late fall and winter, and a heat-tolerant strain which could be colonized in late spring and summer.

Current results with such strains are quite promising. A cold-tolerant strain has been developed which can stand

nearly twice the hours of cold before 50 per cent mortality is reached as could the original; and a heat-tolerant strain has been developed which can stand more than twice the hours of heat before 50 per cent mortality is reached as could the original. This should be more than sufficient to enable these strains to survive and reproduce where the original one failed. Field tests to demonstrate this will be conducted as soon as it appears that the maximum tolerance to cold and to heat of which the species is capable, has been developed in the two strains. Currently, therefore, it appears hopeful that the periodic colonization method can be adapted for use throughout the interior citrus areas.

Meanwhile, as this study progressed, the California red scale has been spreading in the Central Valley of California, where it formerly did not occur. It is extremely bad now on citrus and other host plants in such cities as Bakersfield, Tulare, Fresno, and Reedley, and is being found here and there in commercial citrus. There is little question but that this scale will become the major citrus pest in the Central Valley within a relatively short time. The climate of this area most closely resembles that of the interior citrus areas of southern California, hence it is not to be expected that *Aphytis* will do well unaided against the red scale in the Central Valley. Past experience and observations indicate that periodic colonization of *Aphytis* by commercial insectaries would offer the best likelihood of achieving biological control, especially if high- and low-temperature-tolerant strains are available. Hence, large-scale application of the method could develop in this major citrus area.

Similarly the California red scale is becoming more common in certain eradication districts such as the Coachella-Imperial Valley areas and Ventura County. Should it become generally distributed and abundant, commercial insectary production of *Aphytis* might offer the means of achieving biological control here also.

THE HOST PLANT

Hosts tested included the pink, gray, and orange varieties of banana squash, *Cucurbita maxima*; the citron or cow melon, *Citrullus vulgaris*; the butternut squash, *Cucurbita moschata*; the White Rose, Russet, and Bliss Triumph varieties of commercial potato tubers; and fruit of the grapefruit and lemon.

Evaluation of Suitability. Factors which need to be evaluated in order to determine the relative suitability of a host plant for use in the commercial mass culture of a diaspine scale and its parasite include:

1. Year-round availability: The ideal host plant should be locally available on a year-round basis, either through continuous production or through storage.

2. Cost: Cost should be held as low as possible, but in addition to cost per pound or other unit, such factors as keeping quality and number of parasites produced per unit must be taken into account.

3. Storage requirements. The type and cost of the storage area required must be considered.

4. Ease of handling: Host material should be easy to handle, both in the storage area and in the insectary.

5. Relative suitability to the host scale and the parasite: The plant should be one on which scales reproduce well and develop to near-maximum-size adults.

6. Susceptibility to disease: The incidence of disease, such as rotting, in the host plant should be low and preferably subject to control.

7. Susceptibility to insect pests: The susceptibility of the host plant to unwanted infestation by insectary pests and the ease of control of such infestations is an exceedingly important consideration.

8. Favorability of surface:volume ratio: The ratio of surface area to cubic volume should be as high as possible. The smaller the plant unit the higher the surface:volume ratio will be; hence more scales can be supported in a given amount of insectary space.

9. Length of useful life: The length of useful life of the host plant should extend for 6 months to a year. This will provide for storage periods when the host plant is not available in the field and will insure sufficient time for development of scale and parasite (1½ to 3 months) on a given plant.

10. Special requirements: Certain host plants may require special techniques or treatment for use in the mass-culture method. These must be considered in any evaluation of the relative efficiency of the plant for such use.

Past Work. In early attempts to develop a mass-culture method for *Aphytis*, Bartlett and Fisher (1950) compared relative oviposition activities by *Aphytis* on red scale on citron melons, grapefruit, oranges, and potato tubers when the parasites were free to move from one infested host plant to another. They found that *Aphytis* preferred the above host plants in the order named. Further tests comparing oviposition on red scale on grapefruit with that on potato tubers showed that *Aphytis* laid over three times as many eggs on grapefruit as on potatoes where an equal choice was given. In addition, they found that on potatoes over 92 per cent of the parasite eggs were placed on second-instar scales. This results in small parasites with a low reproductive potential. They considered citron melons and banana squash to be unsuitable for use at relative humidities in excess of 40 per cent, so did not pursue further tests with these host plants, because such a humidity is too low for satisfactory parasite culture.

Flanders (1951), in succeeding work on the same project, chose the White Rose potato tuber as the principal host for the mass culture of *Aphytis*.

Host Plants Rejected as Impractical. For use in a highly efficient continuous mass-culture method, certain hosts of the scale can be eliminated without tests. On the bases of year-round availability and cost alone, only banana squash and potato tubers can meet commercial mass-culture requirements. Citron melons are not commercially available and in any event have no advantages over banana squash for *Aphytis lingnanensis* mass culture. Lemons are too expensive, cannot be stored for as long a period as squash or potatoes, and require special handling techniques. Grapefruit are not available on a year-round basis and are unsuitable for *A. lingnanensis* when they become old.

Comparison of Potatoes and Squash. Inasmuch as potatoes and banana squash were indicated as being the only plant hosts commercially practical for use in the mass culture of *Aphytis lingnanensis*, comparative tests or evaluations were made of the suitability of the two host plants, with attention given to each of the factors previously considered important in such an evaluation.

From the standpoint of insectary production, two factors of signal importance stand out:

1. Suitability as scale host: It has been recognized since 1948 that banana squash is a preferred host for red scale; that it

is a preferred host for oleander scale, *Aspidiotus hederae* (Vallot) has also been recognized for several years. In the insectary, dense oleander-scale populations are required for economic parasite production; such infestations are readily achievable on banana squash while they are difficult or impossible to achieve on potato tubers. This is due solely to the fact that oleander-scale crawlers do not settle well on potato tubers. Even moderately heavy infestations can only be achieved through excessive use of scale crawlers in the infesting process.

2. Suitability as substrate for parasite activity: The amount of oviposition (and therefore the per cent parasitization) is drastically reduced when potatoes are used as the host plant. This is true of both red and oleander scale, though the cause remains obscure. As has been pointed out, Flanders, and also Bartlett and Fisher, observed a similar reduction in parasitization by *Aphytis chrysomphali* of red scale grown on potato tubers. Presumably, the scales, due to nutritional responses, are less suitable to the female parasite as an ovipositional site. The phenomenon may also be due in part to the covering of the margin of the red scale by the potato cuticle, as noted by Bartlett and Fisher.

These factors alone could tip the balance in favor of banana squash as a host plant for a mass-production program. Other important factors are involved, namely:

1. Cost: Initial costs of squash and potatoes are approximately the same, but losses due to cullage of potatoes, both in preparation for insectary use and later, increase the cost substantially.

2. Storage: The storage of banana squash is considerably less expensive than of potatoes, in that refrigeration is not required. Squash can be stored in any roofed storage area.

3. Ease of handling: Squash require little or no culling when being prepared for insectary use, while potatoes must be sorted individually and bad ones discarded. A substantial saving in man-hours results. Squash require no fumigation while potatoes require treatment with methyl bromide for 2 hours at 80° F in order to exclude incipient pests. In preparing potatoes for fumigation during winter months, a warm-up period is required which again takes time and heated space.

4. Susceptibility to disease: Both squash and potatoes are susceptible to certain fungus rots. However, careful selection and handling minimize this difficulty when squash are used.

5. Susceptibility to insect pests: Potatoes are subject to infestation by tuberworm (chief reason for fumigation) and mealybugs. No known insect pests occur in the insectary on banana squash. Phytophagous mites occasionally build up on squash, but these do not cause enough damage to require control.

6. Surface-volume ratio: The surface-volume ratio of banana squash is poor in comparison with that of potatoes, but this lone adverse factor is outweighed many times by advantageous qualities.

Squash Variety. The variety of banana squash to be used is important. The pink has proved to be most satisfactory, although the so-called gray or blue can be used. The orange proved to be unsatisfactory because scale crawlers would not settle well on it, presumably because of its very thick barklike skin.

Squash Size. The size of banana squash varies greatly in the field. From the standpoint of handling, storage, insectary space requirements and favorability of the surface-volume ratio, the smallest squash are theoretically most desirable, but certain concessions must be made to larger sizes in order to obtain enough material from a given field or

market. A range in weight of between 6 and 14 pounds and in length of between 14 and 22 inches was found to be satisfactory in the method used. The maximum acceptable diameter was 7 inches. Squash of such dimensions are usually readily available during most of the growing season in southern California in spite of the fact that the jumbo pink variety is nearly always planted. Special arrangements for culturing the small pink variety can be made with some growers.

Squash Availability, Price, Selection, and Handling. Squash are available in the field from late May or early June through September or early October. The early squash may be obtained in the Palo Verde Valley area around Blythe. The market value of these early squash is high, ranging to $80 or $90 per ton. The price drops rapidly, however, and by mid-July, when San Diego County squash is mature, the price will drop to $50 per ton or lower. By late September, when Los Angeles County squash has been on the market a short while, the price usually reaches its low of around $25 per ton. It then climbs steadily but slowly until late fall or early winter, when only stored squash are available. Purchase of squash, based on a hypothetical use of 10 tons per year, would be as follows:

Area	*Period*	*Tons purchased*	*Total cost*
Palo Verde Valley	Late May-early June	2	$170
San Diego County	July-Aug.	2	100
Los Angeles County	Sep.-Oct.	6	150
	Total		$420
	Average per ton		$ 42

Enough squash must be purchased during the fall for storage to supply needs until June of the following year.

In order to protect and maintain maximum production, it is highly desirable to replenish the host plant as early in the spring as possible, in spite of the high market price which usually prevails at that time. This is true because of some unexplained physiological change in the nature of the squash as it becomes old, which presumably makes it less desirable to the scale as a food source. This phenomenon manifests itself in late spring, when it becomes difficult to obtain a scale infestation of the proper density on old squash. The crawlers placed on such squash fail to settle properly so that many of them leave the squash and are lost completely. A second difficulty with such squash is the very low production of crawlers by the scale which do survive on them.

The parasite production from old squash suffers parallel misfortunes. Owing to drastically reduced scale densities, fewer scale are available for parasitism and increased squash breakdown reduces the number of squash surviving long enough to permit completion of parasite emergence.

It should be pointed out that these difficulties are not characteristic of squash alone, but rather are characteristic of most host-plant materials, including potato tubers. The simplest solution is to acquire fresh host-plant material, regardless of price.

It is preferable to obtain squash directly from the field and transport it by truck to the insectary. In this manner insectary personnel can choose the most suitable squash, and handling and consequent bruising is minimized. A layer of straw is placed in the bed of the truck to reduce bruising of the bottom squash against the floor. In selecting squash, certain properties must be borne in mind. Only solid, well-matured, disease-free squash will hold up well under insectary conditions. It is, of course, impossible to know whether or not a squash is disease-free. Through careful selection, however, squash which are solid (thick-meated) and mature are chosen. Such

squash should be rigid enough to withstand maximum free-arm pressure without noticeable compression; that is, pressure applied with the arms while holding the squash between the palms.

Storage Requirements. The storage area for squash must be cool and dry and protected from frost. This area requires careful planning in order to make maximum use of the volume of the storage facilities. For holding squash, a simple rack approximately 34 inches deep and accessible from both sides, with shelves approximately 18 inches apart, serves the purpose well. Shelves run from floor to ceiling and are rigidly built of dimension lumber in order to hold a heavy squash load. The storage area required is dependent upon the scope of the operation. Each ton of squash stored requires approximately 21 square feet, half of which is utilized in access ways. A sheet-metal-covered lath-house has served well for the purpose at this laboratory. This area occupies approximately 800 square feet and holds approximately 40 tons of squash, the maximum required for use in a three-man insectary from mid-October until the end of the first week in June.

Disease Control. Certain steps need to be taken in order to reduce the incidence of diseased squash in the insectary. Few diseases attack squash in storage other than fungus rots which, for the most part, need a portal of entry. Therefore, it is of the utmost importance that the squash be handled with care in order to eliminate bruises or cuts. Still some loss can be expected and this loss may run as high as 10 per cent. It should not exceed this value if normal precautions in selection and handling are observed.

Beyond the steps already outlined (care in handling), the residue from a decayed squash should be cleared away and the area cleaned. Accurate temperature and humidity control will assist materially in limiting the incidence of disease. The temperature in the scale-production area should be maintained at 75° F with a relative humidity of 50 to 55 per cent. Such conditions are not particularly conducive to rapid fungus proliferation, so that the number of squash lost to decay is minimized.

THE HOST SCALE

Several diaspine scales were considered and evaluated as possible hosts for the mass culture of *Aphytis lingnanensis*. The group used in preliminary tests included two strains of oleander scale, *Aspidiotus hederae* (Vallot); California red scale, *Aonidiella aurantii* (Mask.); San Jose scale, *Aspidiotus perniciosus* Comst.; and latania scale, *Hemiberlesia lataniae* (Signoret). Of these, only the California red scale, San Jose scale, and the two strains of oleander scale were at all suitable.

Host Scale Requirements. Factors to be considered in the evaluation of the efficacy of a host-scale species include ease of culture, length of life cycle, reproductive capacity, presence or absence of males, presence or absence of prolonged molt stages, degree of preference of the parasite for the scale, favorability of the scale in regard to parasite sex ratio, size, and reproductive capacity (prolificacy).

The host scales should grow vigorously on the host plant, attain a near maximum size for the species, suffer little mortality during growth, and produce numerous vigorous progeny which establish themselves readily when transferred to new host plants. If these conditions are all favorably met and manual culture techniques are not complicated, then culture of the host insect is easy.

The length of the life cycle is of no great consequence, except that the longer the life cycle the greater must be the size of the over-all scale culture in order to maintain constant daily production, because a larger portion of the host culture must be held under storage conditions awaiting scale maturity.

The presence of males in a host-scale species is detrimental in the production of *Aphytis*, because they are not desirable hosts (small parasites with poor reproductive abilities result), yet males usually constitute about one half of the progeny of biparental species and occupy considerable space on the host plant, thus reducing by about 50 per cent the number of female scales suitable for parasitization.

The presence of prolonged "molt" stages (that is, stages when the scale body is closely attached to the dorsal and ventral scale coverings) is undesirable because *Aphytis* does not oviposit in scales unless the body is free from the scale covers.

The degree of preference of the parasite for the scale is manifest by the proportion of scales which receive parasite eggs. Obviously the higher the per cent parasitization, the more efficient the method.

Along with a high per cent parasitization by *Aphytis lingnanensis*, a favorable sex ratio of the parasites is desired, inasmuch as this species has males which are of no consequence in field colonization so long as sufficient numbers are present to insure fertilization of all females. For instance, a culture method resulting in a ratio of 8 females to 2 males would be twice as efficient as one resulting in a ratio of 4 females to 6 males, though both methods produced the same total progeny. Either of these ratios can be obtained with *A. lingnanensis* according to the host plant and host scale used for culture.

The closer parasite size approaches the maximum for the species the better, because longevity and reproductive ability are correlated with size of the adult, which in turn is correlated with the size of the immature stages and the nutritional qualities (often size) of the host scale. Host scales, therefore, should be of the maximum size attainable. This depends mainly upon the host plant and degree of crowding of the scale infestation.

Comparison of Host Scales. An evalution of the potentialities of oleander scale as against the red scale in the mass-culture program was undertaken because (1) the latter on any host plant produces unwanted males, (2) it has molt stages which greatly limit the period during which it is susceptible to parasitization by *Aphytis*, (3) when grown on potatoes the per cent parasitization is too low, and (4) the parasite sex ratio is not advantageous.

Bartlett[5] first suggested trying oleander scale, inasmuch as he had observed good parasitization by *Aphytis* on oleander scale in small laboratory cultures. Flanders (1951) considered this species to be unsuitable because of its white scale covering, which obscures incipient infestations of mealybugs, and its susceptibility to attack by the predaceous mite *Hemisarcoptes malus* (Shimer). Inasmuch as the potential mealybug problem is less on squash than on potatoes, and in any event insectary pests might be controlled, we pursued the trials with oleander scale.

There are two sympatric strains of oleander scale (perhaps sibling species —DeBach and Fisher, 1956); one of these is uniparental, that is, consists of females only and reproduces parthenogenetically (without males); and the other biparental, that is, both sexes are present and males are necessary for reproduction. The biparental strain was tried by Bartlett but has the disadvantage

[5] Bartlett, B. R. Personal communication.

of producing about 50 per cent male progeny. On this basis we preferred to use the uniparental strain but ran comparative tests on both, in case there should be other differences which *in toto* would favor the biparental strain. Comparisons were then made between these two and the red and San Jose scales. An evaluation of certain critical factors is given in table 1.

From table 1 it is evident that either strain of oleander scale is decidedly superior to red scale, superior to San Jose to a lesser degree, and about equal in comparison one with the other, except for the unwanted presence of males in the biparental strain. The uniparental oleander strain, therefore, is to be preferred.

DeBach and Fisher (1956) have shown the uniparental oleander strain to be superior to the biparental strain in several biological responses, which determine ease of culture. Crawlers of the uniparental strain showed superior survival at 65°, 70°, and 75° F. Neither strain survived at 80° constant. Survival of the developing scales was markedly superior in the uniparental form at 70° and 75°. The uniparental form produced 107 crawlers per female at 75°, the biparental form only 15. The optimum rearing temperature was 75° for the uniparental scales, but only 65° for the biparental ones. This last difference in itself is enough to preclude the use of the biparental form for mass culture.

Difficulty was experienced in maintaining satisfactory cultures of the uniparental strain until biological studies showed the optimum temperature for culture of this strain to be about 75° F. Normally, insectary temperatures are maintained at about 80°. This cannot be done with the uniparental strain of the oleander scale during its developmental and reproductive periods.

Graph 1 summarizes certain of the important characteristics considered in comparing the life cycles of California red scale on potato tubers with oleander scale on squash under insectary conditions. It will be noted that the red scale begins to produce crawlers on about the forty-first day while it requires approximately 58 days for oleander scale. As pointed out earlier, this means only that a somewhat larger portion of the culture must be held under storage conditions to permit scale growth and maturation.

Table 1. Comparison of the four diaspine scales tested on banana squash for use in the mass production of Aphytis lingnanensis

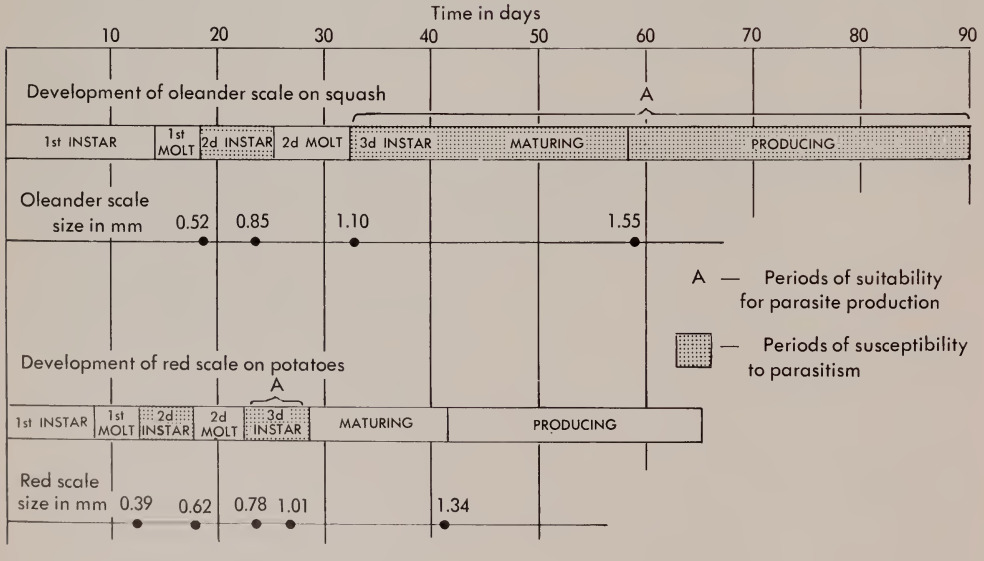
Factors compared	California red scale	San Jose scale	Oleander scale	
			Biparental strain	Uniparental strain
Unwanted male scales present	Yes	Yes	Yes	No
Unsuitable molt stage present	Yes	No	No	No
Relatively high degree of parasitization	No	Yes	Yes	Yes
Per cent of female progeny of parasites	55	67	80	80
Size of parasite progeny	Small	..	Large	Large

The period of crawler production (called "Producing" in graph 1) is extremely variable in both scales. This variability is due not to the scale but to the host plant in both cases. Individual red-scale-infested potatoes or oleander-scale-infested squash are capable of producing crawlers for much longer periods than those indicated in the graph, but an average production period is shown. Because no separately produced mother culture is maintained when oleander scale is used, and because only those crawlers are utilized that are produced during the first 15 days of crawler production (during the first peak of crawler production), extended periods of production become unimportant to maintenance of the host insect when oleander scale is utilized.

Both scales pass through similar growth and molt stages. But, after oleander scale becomes free of its scale covering upon leaving the second-molt period, it never again becomes firmly attached to this covering and is continuously susceptible to parasitization until it dies or becomes so completely expended as a result of extended crawler production that it no longer forms a suitable site for egg deposition by the parasite.

Both scale species first become susceptible to parasitization during the second instar. Even though suitable, however, this scale stage is not desirable for parasite production because the small scale size results in small parasites having reduced reproductive potentials.

With red scale, the third-instar stage lasts for 5 or 6 days. This is the final period in which this scale is susceptible to parasitization, and such scales reared in the insectary are suitable for parasitization only during the middle 4-day period of this third instar. It is for this reason that timing is so important in a program which utilizes red scale. The difference in the amount of time during which the two scales are suitable for parasite production is shown in graph 1. Oleander scale is seen to provide a strik-



Graph 1. Developmental stages, periods, and sizes; and periods of susceptibility and suitability of California red scale on potato tubers and oleander scale on squash to parasitization by Aphytis lingnanensis Comp.

ing advantage. (The terms "suitable" and "suitability" as used here describe those times within the periods of susceptibility to parasitization during which the scale, because of sufficient size, is satisfactory for parasite production.) The sizes of the scale at critical points during its life cycle are also shown.

Parasite Production from Red Scale and from Oleander Scale. No attempt has been made to compare parasite production from red scale with that on oleander scale grown on the *same* host plant since neither red scale on squash nor oleander scale on potatoes permits economical production.

When grown on squash at relative humidities in the 50 to 55 per cent range, many developing red scales in the population are lost because of excessive flow of plant juices at the puncture wound inflicted by the mouth parts. This "gumming" of the squash surface interferes with the over-all production program not only through the loss of some scales, but through loss of crawlers by trapping, and through the encouragement of fungus activity on the surface of the squash with consequent excessive squash breakdown. In contrast, oleander scale on squash produce none of these adverse effects. The reasons why production of parasites using oleander scale on potatoes is not economical have already been stated (see "Comparison of Potatoes and Squash," p. 8). For these reasons the comparison of parasite production from the two host scales is based on data with each scale on its most favorable host plant.

The extent of superiority of parasite production from oleander scale on squash as compared with the earlier method utilizing red scale on potatoes is shown by relative parasitization on red and oleander scales in the following illustration. Figures used are based upon data acquired during a period of *maximum* laboratory production on red scale on potatoes, and upon the *average* production on oleander scale on squash.

Four trays of red-scale-infested potatoes occupy 8,190 cubic inches in the insectary and will produce 18,000 female *Aphytis lingnanensis*. This represents 2.2 females per cubic inch. Six oleander-scale-infested squash held in a compartment of the oviposition collection unit will occupy 11,390 cubic inches and will produce 210,000 female *A. lingnanensis*, or 18.4 females per cubic inch. This represents an increase in production per unit of insectary space by a factor greater than 8. Other equally striking comparisons could be drawn, but this one serves to show the extraordinary parasite production obtained on oleander scale on banana squash.

Biology of Uniparental Oleander Scale. The uniparental strain of the oleander scale, *Aspidiotus hederae*, reproduces generation after generation without males. No male progeny result when males of the biparental strain are placed with females of the uniparental strain.

Crawler settling and survival in this strain is greatest at 75° F. Survival is increased as relative humidity is increased, hence the highest relative humidity should be used which is compatible with nonspoilage of squash, usually about 50 to 55 per cent. Tests with 600 individually isolated females on green lemons were run to determine length of life cycle and progeny production at three temperatures. The results are shown in table 2.

All these data indicate that 75° F is the optimum temperature for culture of the uniparental oleander scale. At 80° crawlers fail to survive and settle, but mature scales can be used for parasite culture at this temperature.

Under mass-culture conditions at 75° F, 58 $\pm$ 3 days is considered to represent the beginning of effective progeny production. During the next 15 days the bulk of progeny production occurs, although

Table 2. Life cycle and progeny production of uniparental Aspidiotus hederae (Vallot) at various temperatures*

Temperature, °F	Average minimum life cycle, crawler to crawler, days	Average age of mother at first peak of progeny production, days	Average days in progeny production	Average total progeny produced
65	86	102	58	37
70	62	75	48	86
75	49	60	38	94
80	Crawlers fail to survive and settle.			

* Average relative humidity 60 per cent; 200 individually isolated females used at each temperature.

on any given squash some production may continue for another month or more. For purposes of obtaining crawlers for reinfestation of new squash, mother scales should be used between the 58th and 73d days of life. After this period the scales are still suitable for use in rearing *Aphytis lingnanensis*.

Newly emerged crawlers are positively phototropic. This reaction is used in collecting crawlers for infesting new squash. The technique is described later. Most crawlers settle, insert their mouth parts and begin feeding within 24 to 48 hours after emergence. If not satisfactorily established on the squash within this period, they usually die. It is highly important, therefore, to collect crawlers for reinfestation of new squash at least every 24 hours.

THE PARASITE

Aphytis lingnanensis has proved to be the most effective red scale parasite in the field in southern California (DeBach, 1954) and easier to culture in the laboratory than its closest competitor and near relative, *A. chrysomphali*. For this reason the mass-culture program was shifted from *chrysomphali* to *lingnanensis* during the early stages of the project.

Biology of Aphytis lingnanensis

Aphytis lingnanensis is ectoparasitic, depositing its eggs usually under the venter of the scale body. The general biology and habits of *lingnanensis* are similar (except for male production) to those of *chrysomphali* described by Quayle (1910) under the name *A. diaspidis*.

Reproduction and Sex Ratio. Mating is necessary for continued female production in *Aphytis lingnanensis*. Newly emerged females mate within the first 4 hours, if males are available. Oviposition by either virgin or mated females begins within 4 to 12 hours after emergence. Virgin females deposit only male (unfertilized) eggs; mated females may deposit either male or female eggs, depending on whether or not the spermatheca is stimulated to release sperm as an egg is being laid. The more favorable the host scale and the environment during oviposition, the greater will be the proportion of females to males. A ratio as great as 9 females to 1 male may be obtained with *A. lingnanensis* reared on oleander scale on banana squash.

Life Cycle. The average duration of the various stages in the life cycle of *lingnanensis* at 80° F and 60 per cent relative humidity is as follows:

Stage	*Number of days*
Egg	3–4
Larva	4–5
Prepupa	1–1½
Pupa	5–6
Total	13–16½

Oviposition. Egg deposition by *lingnanensis* on California red scale on citrus goes on for about 10 days (until death) and averages about 57 per female at 80° F and 60 per cent relative humidity. An average of 5 to 6 eggs per female day is indicated, although the number deposited daily is slightly smaller during the beginning and end of adult life and greater during the middle period. There is considerable variation between individuals, and very definite differences will occur at different temperatures and humidities, on different host plants or on different host scales. The above figures would be considerably greater if the host plant had been banana squash and the host scale uniparental oleander scale. Comparable tests between red scale and oleander scale on squash show over twice as many female parasites produced on oleander scale.

As indicated earlier, on California red scale, oviposition by *Aphytis lingnanensis* is limited to second- and third-instar females, and second-instar and prepupal males. These represent rather restricted periods during the life cycle of the scale. Uniparental oleander scales are susceptible to oviposition during the second instar and from the beginning of the third instar through the adult egg-laying stage, and, in fact, until the female scales are too far spent to support development of a parasite. This period represents a substantial portion of the life cycle of the scale. Usually only one egg is deposited on each third-instar California red scale female, whereas two or more are frequently deposited on third-instar uniparental oleander scale, and two or more parasites may emerge from such scales.

Comparison with Aphytis chrysomphali

At first it seemed surprising that *Aphytis lingnanensis* was superior to *A. chrysomphali* in the field because the former is biparental, needing males for reproduction of females, which is a relatively inefficient process as compared with the uniparental *chrysomphali* which does not produce "useless" males. Comparative biological studies were therefore conducted in an effort to ascertain what advantages *lingnanensis* had which overcame its wasteful production of males. Results are shown in tables 3, 4, and 5.[6]

From table 3 it is obvious that adults of *Aphytis lingnanensis* lived much longer than those of *A. chrysomphali*, at all temperatures tested, but particularly at temperatures above 70° F; in this range, which includes usual insectary temperatures, *lingnanensis* females lived about three times as long as those of *chrysomphali*. (Relative humidity also has a very definite effect on length of life of adults,

[6] These data, for the most part, were acquired by T. W. Fisher, working with the senior author.

Table 3

Average longevity of Aphytis adults at various temperatures*

Temperature °F	Days to 50 per cent mortality	
	Aphytis lingnanensis	Aphytis chrysomphali
50	7	4
60	12	8
70	11	3
80	6	3
90	4	1

* Average relative humidity of 60 per cent.

Table 4

Number of days required for development of Aphytis lingnanensis and A. chrysomphali

From egg to adult at different temperatures and 50 per cent relative humidity

Temperature, °F	Average developmental period, days	
	A. lingnanensis	A. chrysomphali
60	54	45
70	22	22
80	15	14
90	13	..*

* Complete mortality; all eggs failed to hatch.

as will be shown later for *lingnanensis* alone.)

The length of life cycle is an important factor in parasite culture; the shorter the life cycle the more the production that can be obtained in a given period. Comparative life cycles for *Aphytis lingnanensis* and *A. chrysomphali* are given in table 4. At 60° F *chrysomphali* possessed an advantage in that its life cycle is completed more rapidly, whereas at 70° and 80° (normal insectary temperature) the rate of development of the two species was similar. At 90° *chrysomphali* failed to complete its life cycle. This latter probably accounts in part for the superiority of *lingnanensis* in the field.

Table 5

Average number of eggs laid and hosts fed upon by Aphytis females during their lifetime

At 80° F and 60 per cent relative humidity

Activity	Average per female	
	A. lingnanensis	A. chrysomphali
Total eggs	57.1	13.2
Total hosts fed upon	45.9	22.0
Total hosts destroyed	103.0	35.2

One of the most important criteria for comparison is the relative ability of the two species of *Aphytis* to cause mortality of the host. This is dependent upon their reproductive and host-feeding capacities. Host feeding provides the proteins that the adult parasites need in order to maintain continuous egg production. The adult parasite drills into the scale with the ovipositor, secretes a waxy tube around the ovipositor as it is drilling, carefully removes the ovipositor from the hardened tube and then sucks up some of the scale's body fluid through the tube. This results in the death of the scale. Host-feeding activities[7] may cause more scale mortality in the field than does parasitism. The relative reproductive capacity and host-feeding ability of the two species under comparable laboratory conditions are shown in table 5.

The difference in reproductive capacity is highly significant. *Aphytis lingnanensis* deposited over four times as many eggs as *A. chrysomphali*. This confers a striking advantage to *lingnanensis* both for mass culture and in the field. Host-feeding mortality is undesirable under mass-culture conditions, but the ratio of eggs laid to hosts fed upon is much greater with *lingnanensis* than with *chrysomphali*, which is another relative advantage for the former.

Temperature and Humidity Responses

Although it was shown in table 4 that the life cycle of *Aphytis lingnanensis* is somewhat shorter at 90° F than at 80°, enough mortality of immature stages occurs at 90° to make it impractical to use such a high temperature for mass-culture purposes. Adults also die more rapidly at high temperatures. The optimum culture temperature for *A. lingnanensis*, all things considered, is about 80°.

Relative humidity exerts an important

[7] Scales are sometimes killed as a result of drilling and probing without feeding taking place.

influence on survival of *Aphytis lingnanensis*, particularly the adult stage, at practically any temperature. The higher the temperature, the greater is the need of the parasite for an atmosphere having a high moisture content. When given a choice in a humidity gradient, *A. lingnanensis* adults tend to congregate in the area of 90 to 100 per cent relative humidity. Some effects of various humidities on length of life of adults are given in table 6.

From these data it is clear that *Aphytis lingnanensis* adults are very seriously affected by low humidities. At 20 per cent relative humidity they live less than one third as long as at 80 per cent. The extreme effect occurs at high temperature combined with low humidity. These data indicate that at 80° F there is not too much difference in longevity between relative humidity conditions of 50 and 80 per cent. Probably there is actually a greater difference than these figures indicate, as shown by the results between 50 and 80 per cent relative humidity at 70° and 90°. In any case, the relative humidity should be maintained as high as is practicable in keeping with other phases of mass culture. For instance, squash tend to spoil and honey (used as food for parasites) to mold at higher humidities. A relative humidity of about 50 per cent has been found to be the best compromise under mass-culture conditions.

If adult parasites must be held for any length of time, it is evident from table 6 that the combination of 60° F and 80 per cent relative humidity is the best. Storage in test tubes or other containers in a refrigerator is the simplest means of holding adults. The desired relative humidity of 80 per cent can be closely approximated by the use of saturated solutions of table salt (NaCl) in open containers in the refrigerator. Even so, it will be noted that half the parasites will be dead after 22 days under the very best storage conditions. As a rule, storage should not be employed unless absolutely necessary and then only for a minimum of time.

Food Requirements

Newly emerged parasites need a carbohydrate food during their first 24 hours of adult life or they will soon die.

Various tests were run in which the progeny produced per female during a limited period following feeding with different foods was compared. These foods included honey; honey plus MRT (a protein hydrolysate of yeast which has proved to be a valuable food supplement for fruit flies) at dosages of 0.1, 1.0, and 10.0 per cent MRT in honey; honeydews from soft (brown) scale, cottony-cushion scale, black scale, and mealybugs; and no food.

Progeny production was highest on honey alone. The addition of MRT at 0.1 and 1.0 per cent did not increase progeny production, and at 10 per cent there was an apparent reduction. Progeny production on the honeydews ranged from about 25 to 40 per cent of that resulting from feeding on honey. Progeny production in the absence of food (but parasites newly emerged—less than 2 days old) resulted in about 10 per cent

Table 6

Effects of different combinations of constant humidity and temperature on adult Aphytis lingnanensis

As measured by time in days to 50 per cent mortality

Temperature, °F	Days to 50 per cent mortality		
	At 20 per cent R.H.	At 50 per cent R.H.	At 80 per cent R.H.
50	3	7	9
60	5	11	22
70	7	11	18
80	2	6	7
90	1	4	7
Average	3.6	7.8	12.6

of that on honey. This latter figure would be much less if tests had been prolonged, inasmuch as the parasites without food would probably have died within the next 24 hours. Thus, of the foods tested honey best meets the carbohydrate requirements of adult *Aphytis lingnanensis*. It should be constantly available to all parasites from the time of emergence.

Responses to Light

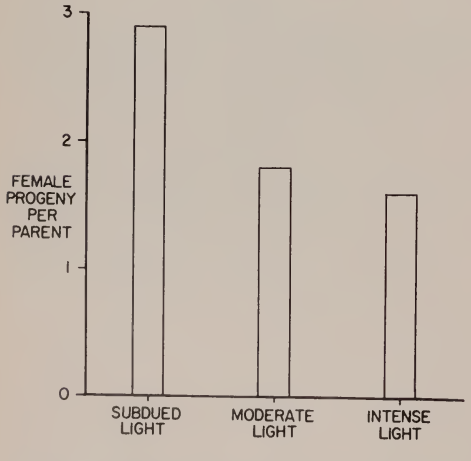
Tropisms of the adult parasite are of importance in mass culture. *Aphytis lingnanensis* females are positively phototrophic; the higher the light intensity, the more attractive it seems to be. Wave length may also be important, since light in the yellow area of the spectrum exerts little influence.

Light requirements for oviposition by *Aphytis lingnanensis* have been investigated. Egg deposition in total darkness is substantially lower than under normal light. In tests run on red scale on potato tubers using a 3-day oviposition period, *A. lingnanensis* averaged 3.3 progeny per parent female in normal light (indirect daylight) and 2 in total darkness.

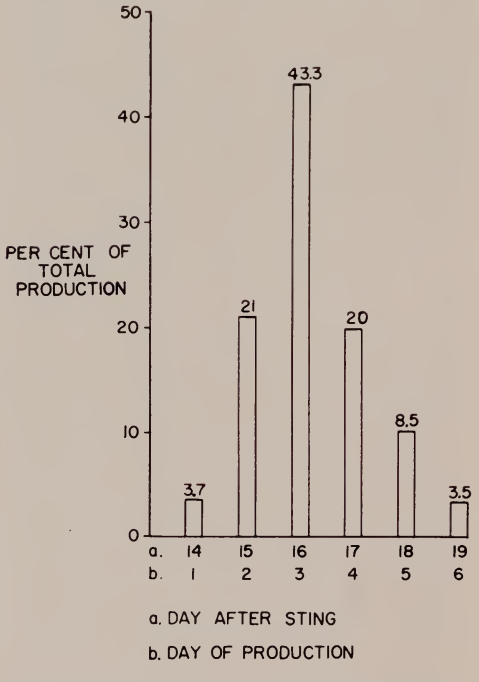
In testing for the effects of light intensity on oviposition by *Aphytis lingnanensis*, parasites were permitted to oviposit on both red and oleander scales for short and long "sting" periods under subdued light (4 ft.-candles), normal light (24 ft.-candles), and bright light (120 ft.-candles). Light-intensity readings were made with the Norwood Director photographic exposure meter, which gives incident light readings directly in foot-candles.

During a short "sting" period (24 hours) on oleander scale, oviposition as measured by progeny per parent female was greatest under subdued light. This is shown in graph 2. During the longer "sting" period, oviposition was apparently unaffected by light intensity.

Under mass-culture conditions, no measurable differences were observed in parasite production in response to light



Graph 2. Effect of light intensity on progeny production by Aphytis lingnanensis Comp. during a 24-hour oviposition period.



Graph 3. The per cent of total parasite production which occurs on each day during the emergence period.

intensity. Some illumination was necessary, however, in order to obtain satisfactory reproduction. Apparently the source of this subdued illumination was unimportant since no measurable differences were observed in oviposition under fluorescent and natural window lighting.

In tests to determine comparative daytime sting rate under natural window lighting vs. nighttime sting rate, oviposition during the 9 daylight hours between 8 a.m. and 5 p.m. was over four times as great as during the 15 hours between 5 p.m. and 8 a.m.

Attempts to increase total progeny production during a 24-hour period by providing light during the entire 24 hours were unsuccessful. Some evidence was acquired which indicated that continuous light during an oviposition period 24 hours or more in duration reduced the total number of eggs laid.

Negative geotropism (the tendency to move against gravity) is also a characteristic of the adult female. Thus, in a closed unit, parasites will tend to congregate on the ceiling and on the side closest to a light source. Position of scale-infested host plants within the closed unit has been planned with this in mind.

Development Rate and Emergence under Mass-Culture Conditions. Under conditions established in the insectary for mass culture of *Aphytis*, parasite development from egg to adult requires 14 to 19 days from the day the egg is laid. Graph 3 shows the average emergence on each day as a percentage of the whole. The emergence peak, approximately 43 per cent of total emergence, occurs on the sixteenth day. In practice, the parasites which emerge on the fourteenth day are collected on the fifteenth along with the emergence which occurred on that day. It is somewhat questionable whether it is worth while economically to collect the small portion (3.5 per cent) present on the nineteenth day.

A NEW CONCEPT OF MASS PRODUCTION

Equilibrium Method of Parasite Production. One of the most important advances in this method and certainly the most revolutionary has resulted from a departure from certain previous philosophies of mass culture. This advance hinges on the new concept that under mass-culture conditions manyfold increase of the parasite per generation in the insectary is not needed. Actually, if production has been built up to 100,000 female parasites per day, for instance, all that is necessary to maintain continuous production at that level is to obtain an average of one female progeny from each mother individual of the orginal 100,000. The mothers can then be immediately collected and colonized in the field, none the worse for having deposited about one egg[8] out of their lifetime potential of 50 or more. This eliminates the need to maintain a large separate mother culture of parasites that lay eggs in the insectary until they die, and hence are lost completely for utilization in the field. The concept has resulted in a much more economical mass-culture method for *Aphytis*.

The maintenance of a constant daily production of the same numbers of parasites by taking only 1+ eggs per female

[8] In the case of biparental, male-producing species, the sex ratio determines the egg deposition necessary per mother. A 50:50 sex ratio would require the deposition of 2 eggs per female, a ratio of 80 males to 20 males, only 1.2 eggs per female. A favorable sex ratio of females to males is therefore highly important. Experience in this laboratory shows that the techniques recommended should yield a ratio of at least 8 females to 2 males. Note that aside from sex-ratio data, all production results, tabular data, recommendations for numbers of sting parasites, for numbers of parasites for field colonization, and other figures, refer to female parasites only.

and then colonizing the females in the field may be likened to what field ecologists call a population in equilibrium. In such a population the number of births is equal to the number of deaths. In the present culture method, equilibrium is maintained by keeping the number of "births" equal to the number of parasites sent to the field after laying their restricted quota of laboratory eggs.

The absolute number of parasites obtained depends merely on the amount of host material, labor, and space used. The success of the method will depend, of course, upon the cost of the parasites in relation to their value in the field.

Flanders (1954) stresses the importance of fecundity, the amount of increase of the parasite population, and environmental resistance. He says:

> The minimum amount of total increase to be expected from an inoculum [of parasites] under economical mass culture happens to be about equal to the number of eggs the average female is inherently capable of producing per day. This is approximately the amount of increase that has been obtained with *Aphytis chrysomphali*....
>
> For the most economical mass culture it is essential to utilize the parental stock [of parasites] to its full capacity. As a rule, the periodic-contact method, a continuous succession of host or prey populations briefly exposed to a permanent parental (parasite or predator) stock population so that environmental resistance is at a minimum, is the most likely method of obtaining the maximum increase possible under mass culture.

Obviously these conclusions do not apply to the present concept. Maximum increase is important only when building initial stocks up to the desired production level. What is important for economical production is to obtain a high degree of parasitization of the densest scale infestation practical. Thus 80 per cent parasitization will double production over 40 per cent parasitization, other factors being equal; and total costs will be cut in half.

The ideal is to obtain 100 per cent parasitization of the host, utilizing the total daily production of the parasite females for "sting" in the insectary for one day (before colonizing them in the field), and to rear from this "stung" material the same numbers of female progeny as female parents. In practice, usually much less than 100 per cent parasitization will be obtained. Based upon the production of female parasites only, the old potato-red-scale method of Flanders (1951) gave no more than 13 per cent parasitization, while the squash-oleander-scale method gives from 40 to 70 per cent.

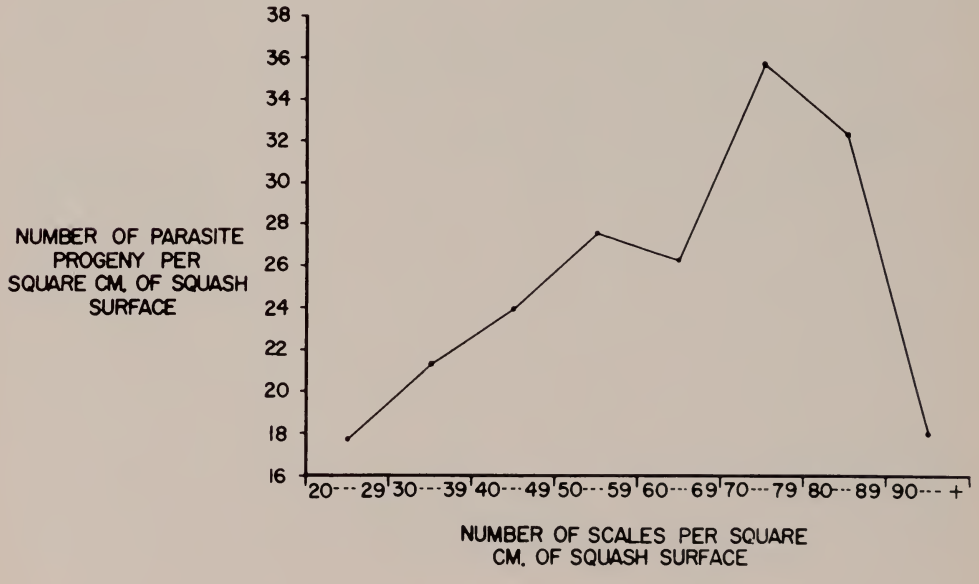
Relation of Parasite and Host Densities. The maximum parasitization obtainable with a given host plant and host scale under given laboratory conditions will depend upon parasite (inoculum) density, host density, and the length of time the two are in contact. The optimum combination can only be determined experimentally.

It is self-evident that the higher the host-scale density the more scales there are available to the parasites and theoretically the more parasites that may be produced per unit area of squash surface. Upper limits to scale population are determined by space requirements—that is, degree of crowding. But parasite production declines when the scale population becomes so dense that individual scales overlap and do not have room to mature normally. Data illustrative of this are shown in graph 4. The tendency for the number of parasite progeny to increase rapidly with an increase in scale density, up to a point, is clear-cut. Maximum progeny production in this case occurred between 70 and 79 scales per square centimeter of squash surface, using an original parasite inoculum averaging 17.5 females per square centimeter. The production of progeny drops off strikingly with a further increase in scale density. Similar trends, differing only slightly in progeny production, occur

when other inoculum densities are used. All data acquired indicate an approximate increase in parasite progeny of 3 for each increase in scale density of 10 per square centimeter up to the point where high scale density results in a decrease in parasite progeny.

In contrast, the use of different parent-parasite-inoculum densities results in very little difference in parasite progeny production, within the limits of the parasite-inoculum densities tested in these studies. If graph 5 is referred to, some indication will be seen of a slight increase in progeny production when higher inoculum densities are used at certain fixed scale densities, particularly 55 and 65 scales per square centimeter. At the lower scale densities differences are insignificant. Hence, within the limits of parasite-inoculum densities suggested for use in mass production (20 to 35 per sq. cm), slight increases may occur, but the point of decreasing progeny production is not reached at any of the scale densities suggested (35 to 65 per sq. cm). Limited additional data indicate that at higher ratios of parasite inoculum to host density, a point will occur at which parasite progeny production per square centimeter will decrease. Such a phenomenon can be explained by the interference of parasites with each other when crowded, resulting in decreased oviposition. This result is not to be expected in the host-parasite combinations suggested herein.

An accurate determination of the optimum numerical interrelations between parasite and host in relation to progeny production is of utmost importance in mass culture. Without such knowledge a culture method would be haphazard at best. Efficiency and economy would be reduced, perhaps strikingly, and progeny production could not be predicted with accuracy. Most previous culture methods have not been based upon such experimentally determined information. The current method depends for its successful



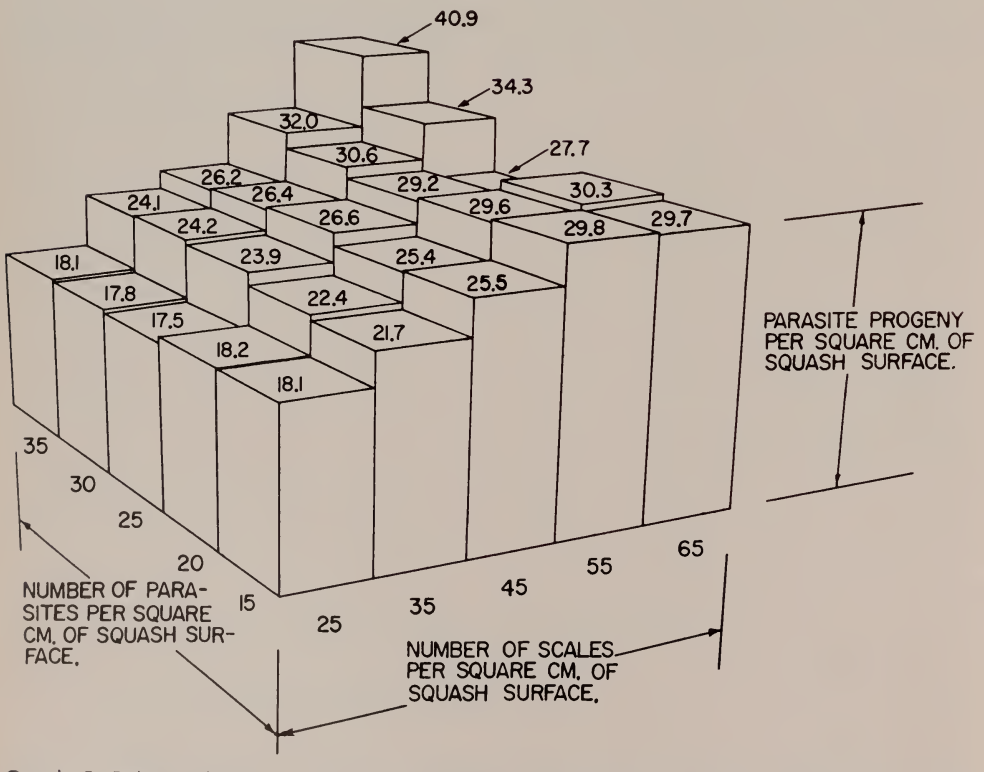
Graph 4. Effect of various scale densities on parasite progeny production using an average parasite inoculum density of from 15.0 to 19.9 females per sq. cm of squash surface (1-day sting period).

and economical operation upon this information more than upon any other single body of data. Graph 5 shows the results of tests utilizing a wide range of combinations of parasite and host densities.

The objective in testing these combinations was to determine which would give a progeny production most closely approximating the desired 1:1 ratio of female progeny to female parents and still give high host utilization.

Study of graph 5 shows that although maximum total progeny production occurs at the combinations involving high host-scale densities, the closest approximation to the desired 1:1 parent-progeny ratio occurs with five combinations—15 parasites to 25 scales, 20 parasites to 35 scales, 25 parasites to 45 scales, 30 parasites to 55 scales, and 35 parasites to 65 scales. This gives a choice of combinations covering all possibilities likely to be used in mass culture.

The first of these (15 parasites to 25 scales) results in a female parasite progeny population of 18.1 per square centimeter. This represents a high degree of host-scale utilization (72 per cent parasitization) but a rather low total progeny production per square centimeter because of the low scale density. The most important consideration is progeny production per square centimeter. The second combination (20 parasites to 35 scales) yields 22.4 female parasite progeny per square centimeter, which represents an increase in total parasite production but a decrease in per cent parasitization to 64. The remaining combinations show successive increments in female progeny production to 26.6, 30.6, and 40.9 per square centimeter. Per cent parasitization declines



Graph 5. Relation between various combinations of inoculum (female parasites per sq. cm) densities and scale densities and resulting female parasite progeny (1-day sting period).

only slightly with these combinations; and anyway, as before stated, production per square centimeter is the important criterion, not per cent parasitization. At any given host density, of course, the higher the parasitization the greater the yield.

All of the last three combinations—25 parasites to 45 scales, 30 parasites to 55 scales, and 35 parasites to 65 scales—would result in extremely efficient mass production of *Aphytis*. If, for instance, the scale density of 55 could usually be obtained, and a parasite inoculum of 30 were used, then 30.6 female progeny per square centimeter could be expected. This would represent an increase in total production (as compared with 25 parasites to 45 scales) of over 35 per cent with no extra effort; hence, a great reduction in cost per acre.

Crawler and Parasite Production from the Same Scales. Another concept somewhat similar to the utilization of the same parasites for both "sting" and field colonization has led to the use of the same individual oleander scales both for production of young scales (crawlers) and production of parasites. This is possible because egg-laying oleander scales are quite suitable hosts for *Aphytis lingnanensis;* hence crawlers can be collected from newly producing scales for about 2 weeks for infesting new squash, and the same scales can then be used for parasite production. This eliminates the need for a separate "mother" stock of scales, which was a necessary extra step in the use of red scale as a host because progeny-producing red scales are unsuitable for parasitization by *A. lingnanensis.*

THE CULTURE METHOD

As previously indicated, the present method for mass culture of *Aphytis lingnanensis* utilizes banana squash (pink variety) as the host plant and the oleander scale (uniparental strain) as the host insect. This combination, together with such improvements in techniques as automatic collection of scale crawlers for infestation of squash, mechanical collection of parasites, development of an oviposition or "sting" chamber to give improved parasitization and isolation from possible pest contaminants, permits efficient and economical production of the parasite. Since success with the method depends upon precision in executing the operations, the techniques are described in considerable detail. But before taking up specific directions, a brief résumé of the procedure may be helpful in obtaining an over-all picture of the method.

The New Method Summarized

Each day a fresh supply of squash is brought into the scale-infesting room from the outside storage area. The next day this batch of squash is infested with scale crawlers collected from squash infested some 8 weeks earlier. The newly infested squash are then transferred to the scale-development storage room, where they are held for 58 days. At the end of this holding period the scales are ready to produce crawlers. Some of the squash are then returned to the scale-infesting room, where they are held 14 days for the collection of crawlers, and then taken to the oviposition-collection room for parasite production. The squash not needed for crawler production go directly to the oviposition-collection room from the storage room. Here the squash are put into the oviposition-collection units, six to a compartment. Honey is supplied for the nourishment of the parasite, the appropriate number of newly emerged *Aphytis lingnanensis* are added to each drawer, and the unit is closed for a day to permit the female parasites to "sting" (oviposit in) the mature scale. The next day the parasites are anesthetized, collected, measured,

and packaged 4,000 to a carton for field colonizing. The squash with parasitized scale remain in the unit until the parasites begin to emerge. On the thirteenth day honey is again supplied. The newly emerging parasites are anesthetized, collected, and measured daily from the fifteenth to the eighteenth or nineteenth day after the "sting" and used for "stinging" a new lot of scale. After the parasites have completed emergence, the squash are discarded. Scale-production operations are diagrammed in figure 5 (p. 29), parasite-production operations in figure 10 (p. 35).

Scope of Operation

In order to understand how the economics of the current method operates, hypothetical insectary operations of various sizes and scopes were subjected to critical analysis so far as cost versus production was concerned. The cost estimates, including man-hours required, and the production potentialities were assessed on the basis of those experienced in this laboratory. As a result of this work, it became apparent that a two-man insectary operation was the smallest operational unit which could be successfully undertaken if the field colonization work were to be handled by insectary personnel and the cost maintained at a level in line with that proposed on pages 5 to 6.

In an operation involving three full-time employees, for example, 30 squash would be stung daily during the major production period of each year between February 1 and November 1, yielding approximately 1,000,000 female parasites daily. Production at this level would serve approximately 700 acres of citrus where maximum colonizations were required, and a still larger acreage if at least a portion of the total were in the coastal climatic zone in southern California, where less extensive releases are required.

During the other three months, from November 1 to February 1, production could be cut back sharply. About one half the peak level could be maintained from November 1 to December 15. Then a gradual increase in production to about three fourths the peak level by mid-January would permit the resumption of full-scale activities on February 1. This cutback period, which coincides with the period during which colonizations are least effective, has two distinct advantages: (1) it is a period when reduced personnel can maintain insectary operations, so that vacations may be taken; (2) it permits a substantial savings in host material and other insectary supplies, including ether and carbon dioxide.

Scale Production

In the routine operation of an insectary, new host-plant material must be infested with the scale insect each day. Squash are selected from the outside storage area, transported to the infesting room, and placed individually on racks the day before infestation in order to permit them to reach room temperature before the infesting process is begun. Dense scale infestations can only be obtained when crawlers settle readily. When placed on a plant surface whose temperature differs markedly from their optimum settling temperature, crawlers will not settle well, and low-density, poorly distributed scale populations result. Even in winter normal infestations are readily obtained on squash which have been subjected to this preinfesting warm-up period. The warm-up period offers no problem, since in routine operation the new squash are brought into the insectary each day as soon as the infesting process for that day has been completed; they then serve as material to be infested on the ensuing day.

Crawler Collection. Crawlers are collected from producing scales by using the "shadow-line" technique. Crawlers on the surface of the producing squash move toward a light source and form a

cluster on the tip of the squash nearest the light. Massed crawlers fall from the tip to a white card below, then move again toward the light until they reach the edge of the shadow cast by "V"-shaped shadow boards. The shadow collector is pictured in figure 1. The line of crawlers can be seen in the lower left center of the photograph.

The light source is a 48-inch fluorescent tube mounted vertically. The shadow cast by this light is not sharply defined, the edge being broad and grading from very light shadow to dark shadow. Such gradation works well and may be superior to a sharply defined shadow in that the crawlers tend to distribute themselves over a broader zone; this reduces the number of crawlers piling up at a sharp shadow line, and possibly thereby reduces injury to the crawlers involved.

The mother-scale-infested squash are arranged on a circular rack as pictured in figure 1, with the crawler collection cards placed below in such a way as to receive crawlers falling from the tips of the squash nearest the light source. A rack constructed in a manner similar to the pictured one, but with three shelves, would accommodate 135 squash, or more than the 120 needed in a two-man operation. The addition of one more shelf makes it possible to accommodate 180 squash, which under normal conditions represents the average number needed for a full-time three-man insectary operation. A rack of this type is pictured in a later section (see "Mass-Culture Equipment," p. 36).

A large collection of crawlers is shown at the shadow line in figure 2. From the card, the crawlers are transferred to a common kitchen-type metal salt shaker. This is done by lifting the ends of the card on which the crawlers have been collected until the ends of the card meet, grasping both ends in one hand with one finger inserted between the edges in order to keep the edges separated, rapping sharply on the side of the roll thus formed with a pencil or the end of the handle of a camel's-hair brush, and pouring the dislodged crawlers into the shaker. (See fig. 3.) Frequently it is necessary to use a 1-inch camel's-hair brush to dislodge some of the crawlers which have not been loosened by the earlier rapping. These, too, are poured into the shaker and the perforated lid of the shaker is replaced.

The Infesting Process and Holding Period. Prior to infesting, each squash is dated with a wax marking pencil. The date is placed on the squash surface, using large numerals, 2 inches or more in height. (Developing scale tend to obscure the date unless large numerals are used.)

Fig. 1. The shadow crawler collector.

Fig. 2. Crawlers collected at the shadow line.

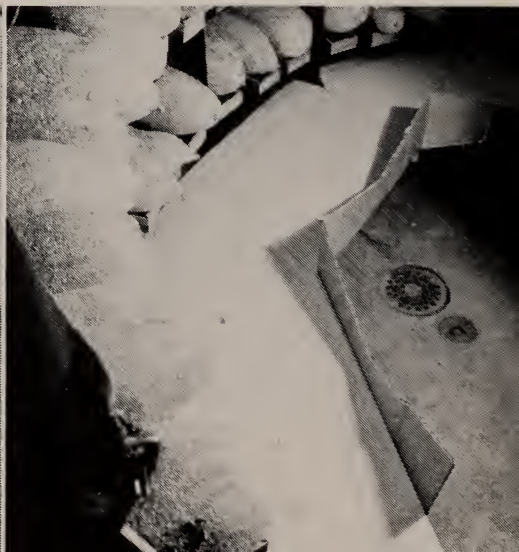


Fig. 3. Transfer of crawlers to shaker for infesting.

Fig. 4. Infesting squash from shaker.

Crawlers are distributed over the upper surface of the squash as shown in figure 4, and given from several seconds to 2 minutes to scatter and spread. A low-angle light source (a 60-watt desk lamp is satisfactory) encourages this spreading. The squash are then rotated about a one-sixth turn away from the light and the newly exposed upper surface is infested in turn. This process continues until the squash has been completely rotated and infested. By infesting squash arranged in groups of from 6 to 10, a rapid, continuous process results.

As has been pointed out in an earlier section, fairly dense infestations—ranging above 35 scales per square centimeter—are required for economical production of parasites.

Proper scale densities cannot be obtained unless certain precautions have been taken to insure an evenly distributed infestation. These include: (1) Immediately after the distribution of crawlers over the surface of a squash, the squash is rotated upon its polar axis and examined for surface areas which have been inadvertently missed. (2) After the surface has been infested completely, the squash is removed to a darkened room where it remains for a minimum of 48 hours in order to permit all crawlers to settle without being attracted to one area of the squash or induced to leave the squash by a phototropic response to light.

After crawler settling, the squash are removed to a room receiving normal daylight but from which direct rays of sunlight are excluded. Under commercial operation, this move might be eliminated if the dark, squash-holding room is sufficiently large. Comparisons of crawler production from scale which matured in darkness with that of scale which matured in a normally lighted room showed no measurable difference.

When the scale on squash in the holding rooms have reached 58 days of age, the squash are removed for use in the production program.

Figure 5 shows diagramatically the typical host-scale handling procedures in a two-man insectary operation. Twenty-one squash per day are infested, all of which go to the holding area for scale maturation. Crawler production at its poorest requires not more than 126 squash in the mother-scale culture and these need to be completely renewed each 14 days, or at the rate of 9 squash daily.

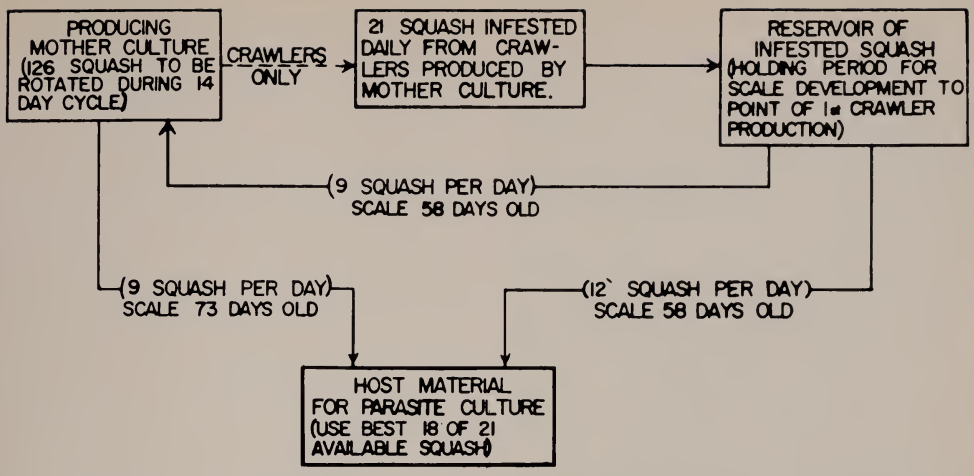


Fig. 5. Diagram of typical handling procedures for scale-infested squash in a two-man insectary.

It follows then that of the 21 squash completing the maturation period each day, 9 are required for the mother culture and the remaining 12 go directly to parasite production. Of the 9 squash completing the crawler production period, the best 6 go to parasite production, permitting a total of 18 squash daily for parasite production. The 3 extra squash allow for loss of poor or spoiled fruits.

Temperature and Humidity Requirements in Scale-Production Rooms. Precisely controlled temperature in all scale-production areas in an insectary is extremely important for two reasons: (1) It is essential for continuity of parasite production to have a uniform and continuous crawler and maturing-scale production. Fluctuating temperatures result in unpredictable scale-maturation periods. (2) The scale, though able to withstand higher temperatures for short periods, does not do well under constant temperatures above 75° F but matures and reproduces well at 75° F.

Oleander-scale crawlers settle and survive best at a high humidity; but satisfactory reproduction, crawler settling, and survival occurs at constant humidities as low as 50 per cent. Because of the susceptibility of banana squash to fungus decay, humidities above 55 per cent are not advisable. Both the scale and the host plant function well in the insectary within the range of from 50 to 55 per cent relative humidity.

Parasite Production

Previous Methods. When red scale on potatoes was used as host material in early attempts to mass-produce *Aphytis lingnanensis*, oviposition occurred while the potatoes were supported on an open rack placed before a window. This method had a number of drawbacks, including the inability to control the density of the parasite inoculum, and the inability to confine the parasites in the proximity of the host scales to insure adequate oviposition.

In a later attempt, red-scale-infested potatoes were stung in a closed unit in an effort to control these factors. This unit method was unsuccessful for two reasons: (1) potatoes, as noted earlier, are an unsatisfactory host, and (2) the unit provided inadequate light and ventilation.

Early attempts to utilize oleander scale on squash showed promise when stung

in the open room at the window. But laborious and inefficient collection of parasites by aspiration, as well as the necessity for accurate inoculation density control, dictated a change to a closed unit, both for oviposition and collection. The ability to control the sting within certain limits in the closed unit made it possible to approximate closely the desired 1:1 ratio between female parent and female progeny.

The oviposition-collection unit. The oviposition-collection unit, as pictured in figure 6, contains 5 compartments, each capable of holding 6 squash. This unit accommodates 30 squash, or the maximum used daily in a three-man insectary operation. A slight rearrangement would permit the addition of a sixth compartment so that one such unit would accommodate 36 squash, the maximum for 2 days' parasite production for a two-man insectary. Structural details and numbers required will be given in a later section, but certain operational details follow.

Each compartment is built on drawer-type slides so that it can be drawn from the unit. Additionally, a lever ending in an eccentric at each end of each unit permits the unit to be lifted approximately ½ inch in order to provide freedom for the base card, which serves as the floor of the oviposition unit and rests on the floor of the drawer assembly. The drawer assembly is pictured in figure 14, A. A portion of the top of the compartment is hinged to provide access, and a sheet of plastic (vinyl acetate) attached to the lower surface of this lid serves as a honeyed feeding surface which is exposed when the lid is open (see figure 14, *A*). Honey is streaked on this surface, as shown in figure 7. The squash in each compartment rests on two rods (⅜-inch galvanized pipe). Finally, glass forms one wall in order to admit ample light, and cloth forms the opposite wall to permit adequate ventilation. Further discussion of the unit will be found in a later section.

Fig. 6. Parasite oviposition-collection unit.

The "sting." Daily during the major production period, in a two-man insectary, 18 scale-infested squash are transferred from the scale-production area of the insectary to the parasite-production area. Six squash are placed in a compartment, honey is applied to the feeder strip, a measured number of anesthetized parasites (the inoculum) added, and the unit closed. This process is repeated until the desired number of compartments are filled.

Determining the Size of the Inoculum. In order to determine how many parasites to add to each drawer of the oviposition unit, it is first necessary to estimate the average scale density on the squash in that drawer.

Figure 8 shows how various scale densities appear on the squash surface. It will be noted that there is considerable open space between scales at and below the density of 40 per square centimeter, and that above the density of 80 there is no squash surface showing at all.

An average scale density of from 60 to 70 per square centimeter of squash

surface should be the goal; but as indicated earlier, such a high density is difficult to obtain. Diligent effort to reach this goal, however, should result in actual infestations on all squash between the desired density limits of 35 and 70.

The recommended inoculum densities and the expected female progeny yield per square centimeter for scale densities of 35 to 65 per square centimeter are shown in table 7. These recommendations are based on the data shown in graph 5 and discussed on pages 22 to 25.

Most of these combinations give a surplus of female progeny over female parents of from 6 to 15 per cent, for an average of about 10 per cent. This will allow for handling losses of up to an average of 10 per cent while maintaining constant peak production.

Fig. 7. Placing honey streaks in parasite oviposition-collection unit.

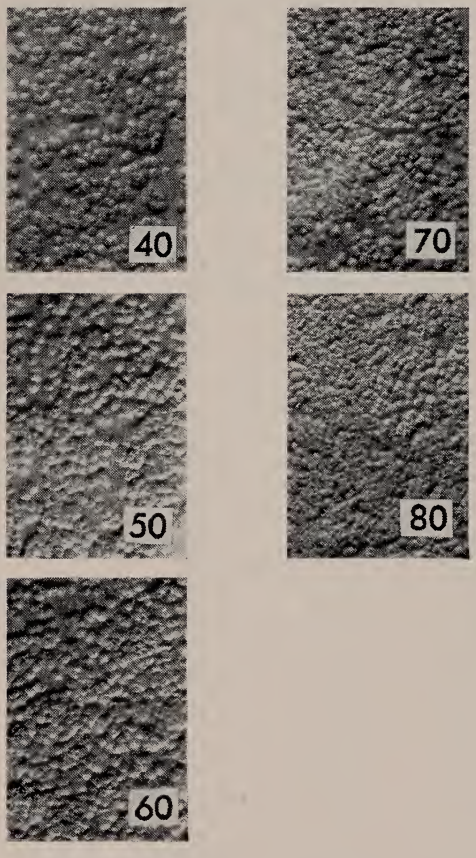


Fig. 8—Appearance of various scale densities expressed as number of scale per square cm of squash surface.

The average surface area of the squash in the drawer must next be estimated. Since surplus squash usually are available, squash should be chosen to obtain the largest size that will permit 6 squash per compartment of the parasite oviposition-collection unit. The minimum size should be 14 × 5 inches, the maximum no greater than 22 × 7 inches. We have used squash averaging about 1,800 square centimeters (18 × 6 inches).

Table 7

Recommended female-parasite-inoculum and host-density combinations and approximate expected parasite progency production

Per square centimeter of squash surface

Estimated number of host scales per sq. cm	Recommended female parasite inoculum per sq. cm	Expected female progeny per sq. cm
35	20	22.4
45	25	26.6
55	30	30.6
65	35	40.9

Table 8 shows the approximate surface area in square centimeters of various sizes of squash.

When the scale density and the squash areas are averaged for the six squash in one compartment of the parasite oviposition-collection unit, then the total number of parent parasites to be added can be calculated by multiplying the appropriate parasite inoculum per square centimeter for the scale density involved (see table 7) by the total squash area in centimeters (see table 8). However, most of the probable parent-parasite inoculum totals necessary for use in mass production are given in table 9, which shows the total female parasite inoculum to be used *per squash* at various combinations of scale density and squash surface area. These figures would of course be multiplied by 6 to get the total for a compartment. The data for measuring parasite numbers by volume are given in graph 6.

Taking a compartment with near-average squash of 1,800 square centimeters surface area and average scale densities of 35 per square centimeter, then 20 parasites per square centimeter should be used (see table 7) or 36,000 (1,800 × 20) inoculum parasites per squash, or 216,000 (36,000 × 6) inoculum parasites per compartment of 6 squash. From this, an average return of 40,300 (22.4 progeny per sq. cm × 1,800—see table 7) female parasite progeny per squash, or 241,800 per compartment of 6 squash, could be expected. If a 10 per cent handling loss is assumed (probably a high estimate) the insectary parasite population will remain in equilibrium (a ratio of one female parent to one female progeny), and a net of 36,300 female parasites will be obtained per squash.

Under efficient mass-culture conditions the insectary average should approxi-

Table 8. Approximate surface area of banana squash which fall within the indicated size range*

Length of squash, inches	Diameter of squash in inches				
	5	5½	6	6½	7
	Approximate surface area, sq. cm.				
14	1,171	1,303	1,426	1,565	1,697
14½	1,208	1,340	1,468	1,608	1,748
15	1,245	1,382	1,524	1,656	1,802
15½	1,282	1,429	1,570	1,702	1,853
16	1,327	1,468	1,619	1,759	1,905
16½	1,363	1,506	1,660	1,815	1,953
17	1,402	1,548	1,707	1,859	2,011
17½	1,438	1,594	1,756	1,911	2,079
18	1,477	1,634	1,798	1,956	2,128
18½	1,514	1,673	1,840	2,008	2,172
19	1,554	1,716	1,891	2,060	2,229
19½	1,594	1,763	1,933	2,110	2,279
20	1,630	1,809	1,980	2,158	2,336
20½	1,672	1,843	2,030	2,209	2,389
21	1,711	1,899	2,069	2,258	2,446
21½	1,752	1,930	2,118	2,304	2,492
22	1,786	1,973	2,169	2,356	2,541

* Computations based on the method of Turrell and Vanselow (1946).

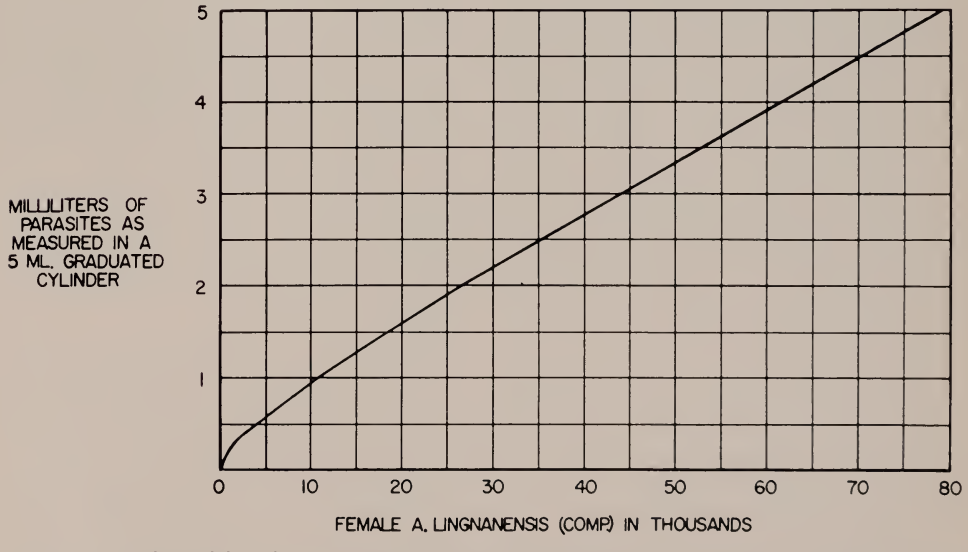
Table 9. Total female parasite inoculum per squash for use at given scale densities and squash surface areas

Squash surface area, sq. cm	Total female parasite inoculum per squash			
	For 35 scales per sq. cm	For 45 scales per sq. cm	For 55 scales per sq. cm	For 65 scales per sq. cm
1,200	24,000	30,000	36,000	42,000
1,400	28,000	35,000	42,000	49,000
1,600	32,000	40,000	48,000	56,000
1,800	36,000	45,000	54,000	63,000
2,000	40,000	50,000	60,000	70,000
2,200	44,000	55,000	66,000	77,000

mate this last figure. Annual cost and yield estimates, which are discussed in a later section, are based upon an average net per squash production of only 35,000 female parasites in order to be on the conservative side.

Parasite Recovery. After an oviposition period of about 22 hours, the parasite inoculum is recovered by anesthetizing the parasites, then rapidly blowing them from the squash surface to the card below, removing the card, and pouring the still anesthetized parasites into a dispensing device (fig. 9) or a temporary storage carton.

In order to anesthetize the parasites, the cloth side of the unit must be covered. This cover is shown on the open compartment in figure 6. Carbon dioxide is forced to flow over anesthetic-grade ethyl ether and admitted to the unit for 2½ minutes at a regulated pressure of 4 pounds per square inch (see fig. 21, p. 42). With the gas still flowing, the lid of the unit is opened, and the anesthetized parasites are blown from the upper surfaces of the squash with the flowing



Graph 6. Number of female parasites contained in a given volume. (Based on a sex ratio of 80 females to 20 males.)

stream of gas by passing the tip of the hose over each squash with a rapid sweeping motion. The gas is then turned off and the unit closed.

Recovery of the parasites is accomplished by raising the compartment (by depressing the lift levers at the ends of the compartment) and removing the base card upon which the anesthetized parasites have fallen. These parasites may then be poured into the dispensing device which is used to measure by volumetric means the number of parasites placed in a release carton.

The Holding and Collection Periods. The squash remain in the compartment. The surplus honey is washed from the feeding surface and this surface carefully dried. The lid of the compartment is propped open as far as possible (about ½ inch with the compartment pushed all the way in). These steps assist materially in keeping the humidity down within the compartment, which, in turn, aids in the control of squash decay.

The unit containing the "stung" squash is then held for 14 days for parasite emergence to begin. On the thirteenth day from the sting date (date of oviposition), honey is applied to the feeder strip and the unit is closed. For four successive days, the fifteenth to eighteenth, the newly emerged parasites are collected. The procedure is similar on each occasion to that outlined above for the recovery of parasites. Graph 3 (page 20) shows the recovery on each day (as a percentage of the total production) experienced in this laboratory. At each collection, additional honey is applied to the feeder strip as required.

These parasites are poured into a graduate and the volume recorded. This volume can be transformed into an estimated number when a 5-ml graduate is used, by reference to graph 6, which shows the number of female parasites contained in a given volume.

The collected parasites are measured and the estimated number is recorded. They are then placed in a well-honeyed 6 × 8-inch animal or battery jar. Each jar is covered with a square of cloth (percale) which is held in place with two rubber bands. These may be stored in a 60° F cabinet in which the relative humidity is held at 75 to 80 per cent, or may be used as soon as desired for parent stock in a new inoculation.

Other Collection Methods Tested. This method of collection is vastly superior to all other methods tried. Aspiration of parasites from the window, an early collection method, was laborious, inexact in that the number of parasites in each tube collected was a difficult visual estimate, and not very efficient in that many parasites remained on the host and were not available for collection. Even the use of additional strong artificial light failed to draw very many of these parasites from the host, so did little to improve the method.

Another collection method which was attempted involved the use of a series of vials mounted over a closed unit. Light was cast on the vials while the parasites within the unit were in subdued light. This arrangement gave very poor results until certain insect repellents were sprayed on the surface of the host-plant material. Of the various repellents tried, which included a commercial product (mosquito repellent), dilute KOH, dilute HCL, dilute acetic acid, dilute carbolic acid and others, dilute carbolic acid gave the best response. At best, however, this method did not approach the efficiency required.

Packaging and Holding Parasites for Field Release. Upon recovery of the parasite inoculum at the termination of the oviposition period, the parasites are distributed into field-release units. Half-pint ice-cream cartons serve well for this purpose. The device (custom-made; see p. 42) for placing an equal

number of parasites in each field-release container is pictured in figure 9. The reservoir is filled with anesthetized parasites. By holding in the hand with a finger under the stem and tilting, parasites are made to fill the stem to the overflow level. Four thousand females (5,000 total, including 1,000 males) fill the stem, which is emptied into a release container, a strip of honeyed paper is added to supply food for the parasites, and the container is closed. It is now ready for temporary storage or transport to the citrus grove.

The honeyed paper is prepared for the release carton by smoothing a very thin layer of honey over a sheet of commercial-grade (heavy) wax paper, covering this layer with another sheet of paper, and rolling with a photographic roller until the honey layer is uniform. This sheet can then be cut into strips approximately 1½ inches wide and 8 inches long. Each strip is composed of two closely apposed strips, which, when sep-

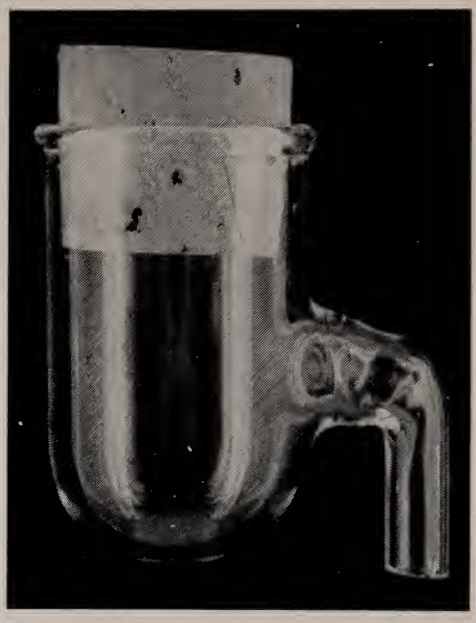


Fig. 9. The parasite dispenser. The lower part of the small tube holds 5,000 parasites, giving 4,000 females with a sex ratio of 80:20. It is filled by tilting from the main reservoir while a finger stops the opening.

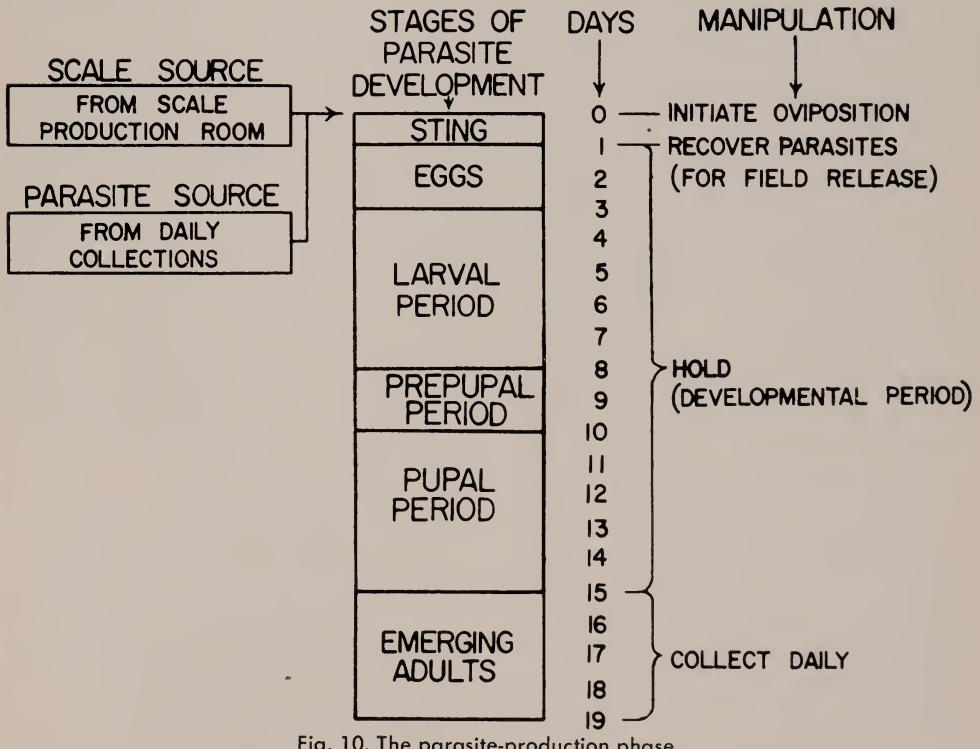


Fig. 10. The parasite-production phase.

arated, serve as two separate honeyed strips. One strip is circled around in the inside of the carton.

All parasites should be released in the citrus grove as soon as conveniently possible after having been cartoned. As indicated earlier, however, storage under the proper conditions at 60° F at a relative humidity near 75 to 80 per cent does no appreciable harm for a period limited to a few days.

Organization of the parasite production work is shown in figure 10.

Temperature and Humidity in Parasite Production Room. Temperature and humidity levels are necessarily a compromise. Previous reference has been made to the fact that *Aphytis lingnanensis* prefers a high humidity at most temperatures. Low humidities, on the other hand, are required to prevent fungus decay of the squash. At 80° F the parasite reproduces satisfactorily when the relative humidity is maintained in the 50 to 55 per cent range. Squash loss in this humidity range is held to a low level, making this the satisfactory compromise of humidity requirements. The temperature used (80° F) is also a satisfactory compromise between lower temperatures which cause longer generations but offer longer adult survival, and higher temperatures which induce shorter generations but reduce adult survival. The time required for a generation of parasites at 80° is 16 days (to 50 per cent emergence of progeny) and no appreciable adult mortality occurs so long as ample food is continuously provided. These conditions (80° F with relative humidity near 50 per cent) are maintained throughout the parasite-rearing area.

MASS-CULTURE EQUIPMENT

In the following discussion, the equipment necessary to the mass-production program is listed and described. Concerning construction, only those details which are less obvious are given and latitude is left for some variation.

The Crawler-Collection Unit. The crawler-collection unit used in this laboratory was semicircular in shape. One large enough to accommodate the necessary number of squash for a two-man mass-production program would be circular, and would require three shelves. A four-shelf unit would be needed to serve the demands of a three-man production unit.

The unit is composed of a series of lesser units. Figure 11 shows the structural details of the basic unit, and these units, when fastened together serially, form the collection unit. Fifteen such units would be required. When fastened together, the completed unit is approximately 18 feet in diameter. A portion of the collector used in this laboratory is shown in figure 1 (page 27). Figure 12 shows a more extensive section of a unit suitable to this method.

The Oviposition-Collection Unit. In any mass-production program following the present method, the parasite oviposition-collection units represent the largest single item of expense. The five-compartment unit is pictured in figure 6. The structural details for the framework of this unit are shown in figure 13. This framework is made of 2 × 2 inch white or Douglas fir. The legs are built up to measure approximately 4 × 6 inches in order to accommodate the heavy-duty rubber-tired casters.

A single compartment from this unit is shown in figure 14, A. Dimensions and structural design are illustrated in figures 14, B and 14, C. The compartment lid is hinged and is fastened with suitcase-type catches. An acetate plastic strip is affixed to the underside of this lid, which serves as a feeder strip and upon which honey is streaked. In order to make

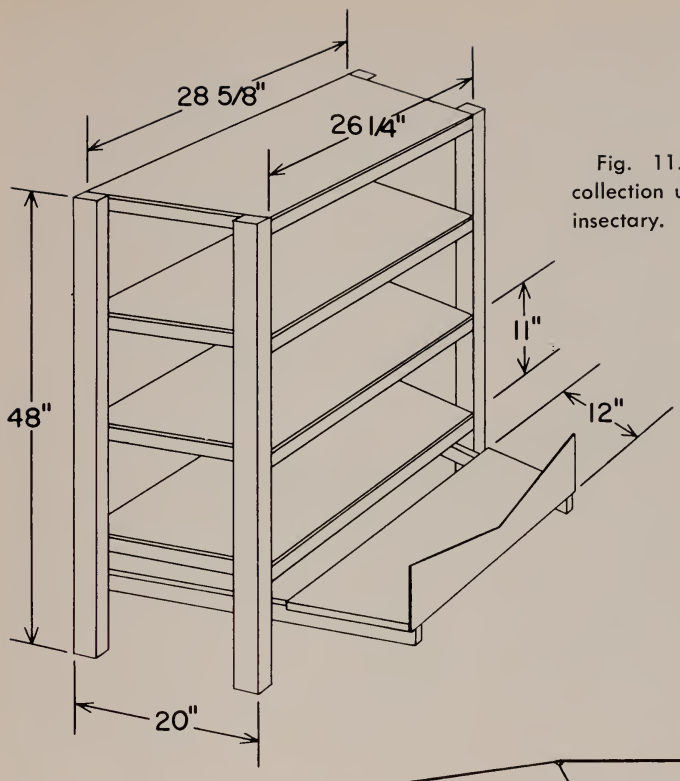


Fig. 11. Basic details of crawler-collection unit required for a three-man insectary.

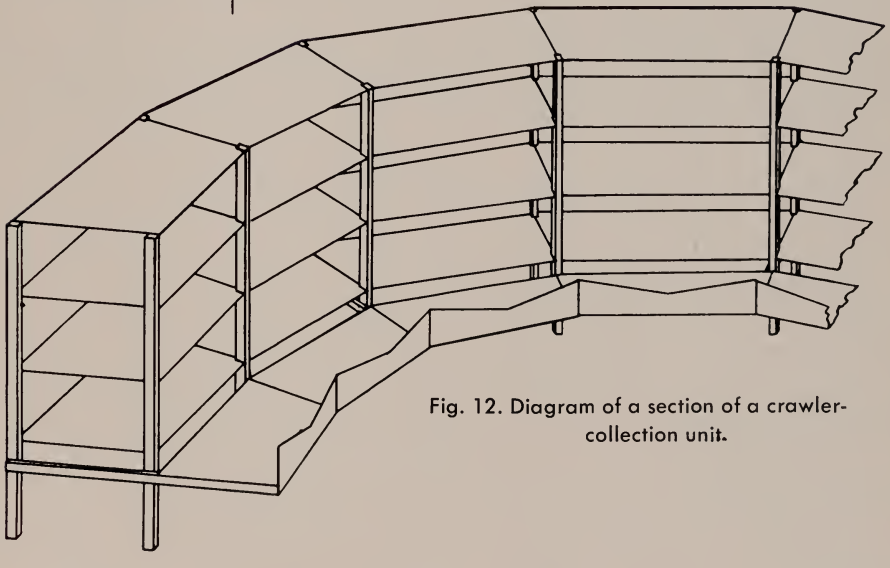


Fig. 12. Diagram of a section of a crawler-collection unit.

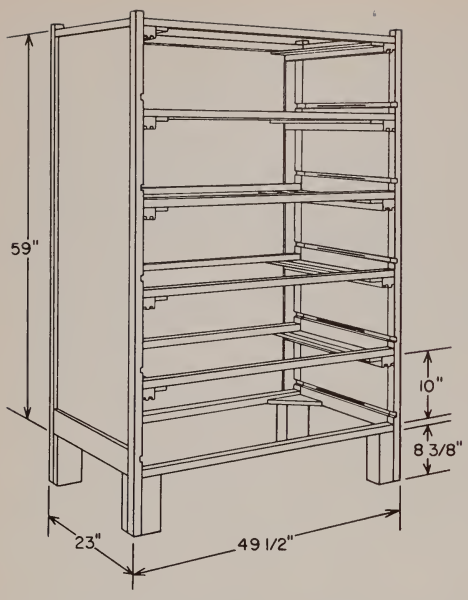


Fig. 13. Structural details of framework of the parasite oviposition-collection unit. Note the runners in each compartment area which serve to guide the drawer assembly.

the lid parasite-proof, rubber door-seal is placed around the three edges on which the lid closes and between the lid and the contiguous portion of the compartment top. (See fig. 14, C.)

The two ends of the compartment are solid. Near the top, midway between front and back, a cleat forms the "handle" against which the eccentric lever exerts its lift. Near the bottom and spaced as indicated in figure 14, B, two rods supported only at the ends are mounted. These support the squash when the compartment is in operation.

No bottom is provided for the compartment. Instead, it rests on a base card which serves as a removable floor. Rubber door-seal around the lower edge of the compartment serves as a parasite-proof cushion for the compartment.

The front of the compartment is cloth-covered, but must be fitted with a solid removable cover which, when in place, makes the compartment reasonably gas-tight. A closely fitting door is adequate for this purpose and no additional effort need be expended to improve the gas-tight characteristics of such a door since it is necessary for the air in the compartment to be displaced during the anesthetization process. A hole is drilled in this removable cover for the purpose of admitting the carbon dioxide and ether mixture during the anesthetizing process. Not more than 10 covers are required if all compartments are standard size.

The back side of the unit is structurally similar to the front side, but is covered with a piece of glass equal in size to the side which it covers and held in place with plastic cement. Size of the glass has been stipulated here since it is very important to keep all inner surfaces of the unit flush and smooth. A smaller piece of counter-sunk glass would serve as well but is a bit more difficult and expensive to prepare.

The drawer assembly which carries the compartment is shown in figure 15, A and B. The bottom is made of ½-inch plywood. Each end is made of three pieces of ¾-inch plywood. The innermost plywood plate is permanently affixed to the drawer, forming the ends of the drawer (as seen in fig. 15), while the outside plate is permanently affixed to the framework of the unit, forming part of the unit's wall. The middle plate "floats" between these and is controlled by appropriately spaced stops.

Mounted just inside these plates are the eccentric lift levers. These levers are made of ¼-inch masonite and are so shaped that the unit remains suspended when the levers are pushed down and does not drop back into place until the levers are raised. Immediately below the levers, and in the corner formed by the junction of the bottom with the inner plate, a spacing cleat which serves three functions is installed. This strip, ¼ × 1 inch, is as thick as the lift lever and (1) maintains the suspended compartment in its correct lengthwise position in the drawer, preventing twisting or binding

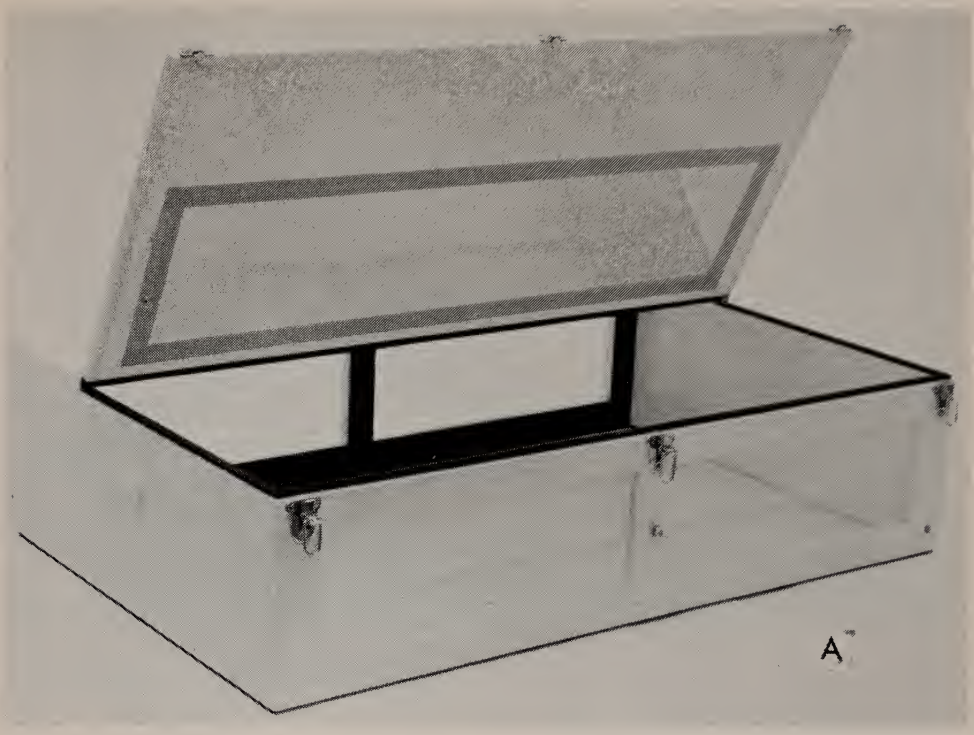
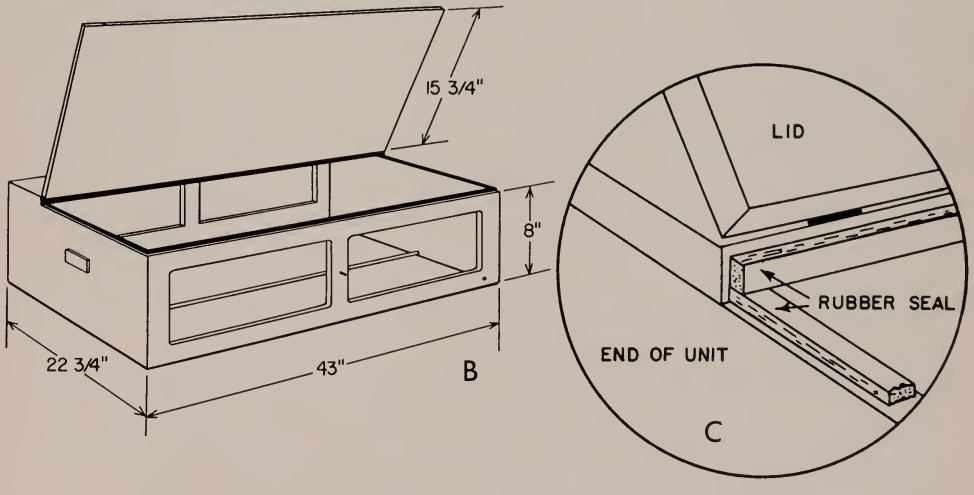


Fig. 14. A, A single oviposition-collection compartment from the oviposition-collection unit; B, details of a compartment; C, detail of parasite-tight seal for lid of compartment.



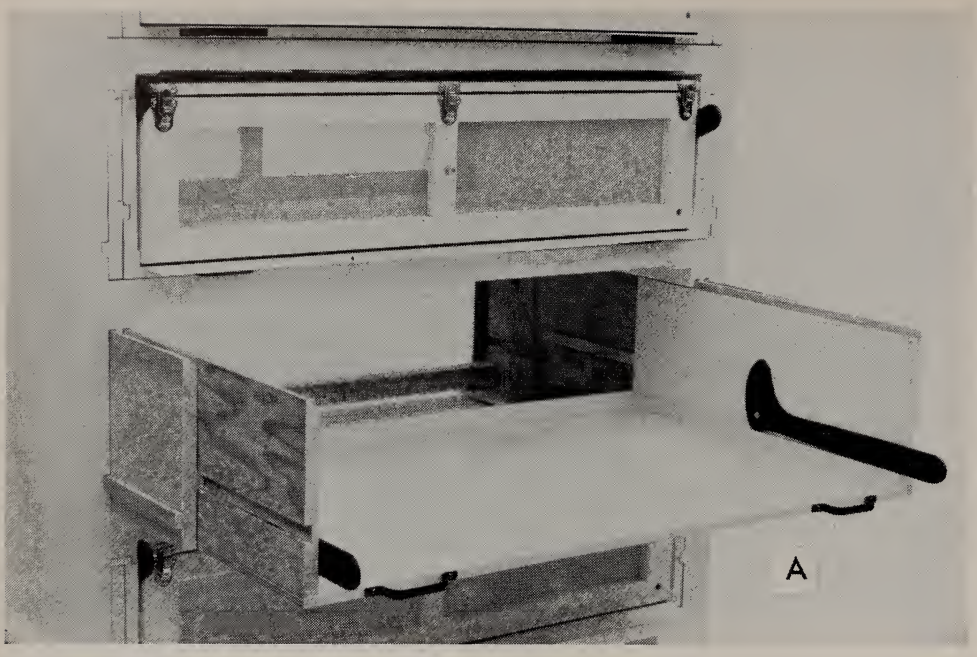
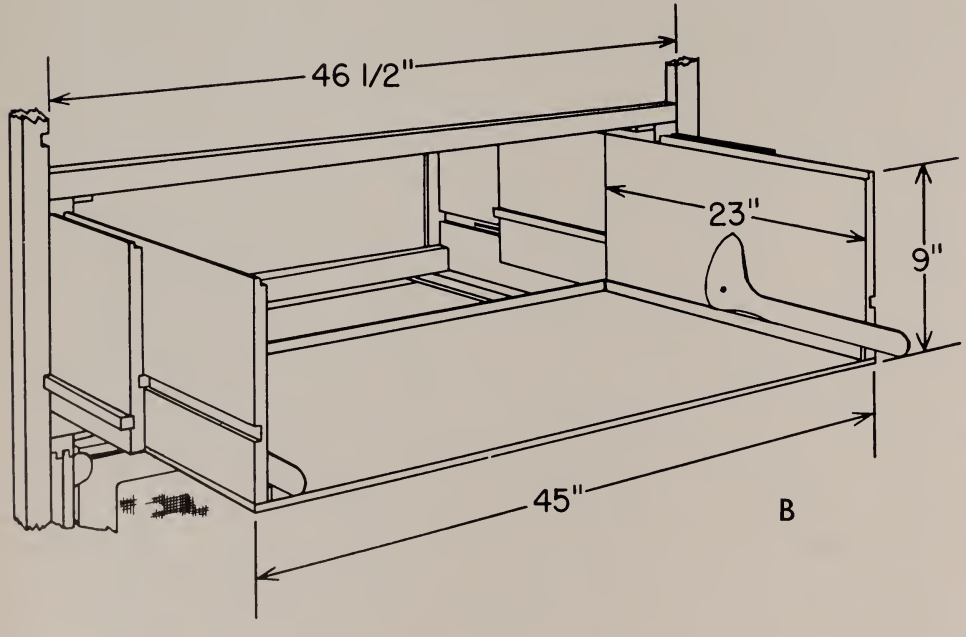


Fig. 15. A, Drawer assembly which carries the oviposition-collection compartment; B, details of drawer assembly which carries the oviposition-collection compartment.



when the compartment is raised; (2) serves as a stop for the levers when these are depressed; and (3) serves to strengthen the corner of the three-sided drawer, since only the inner plate is attached to the bottom.

These ends of the drawer which form the inside plates have a groove rabbeted across them through which a cleat on the "floating" plate travels. On the outside surface of this middle plate, a second cleat travels in a groove rabbeted from the outside plate which serves as part of the unit wall. This slide arrangement permits the unit to carry the full weight of the loaded compartment even when the compartment is fully withdrawn from the unit. Figure 15, B shows structural details and dimensions of the drawer assembly.

In a mass-production program employing two men, 10 such units each having 6 compartments are required. In a three-man operation, 20 units each having 5 compartments are required. These numbers allow for one spare unit plus one unit required in the rotational operation of the parasite culture. Price and depreciation of these units will be discussed in a later section.

Squash-Holding Racks and Trays. Figure 16 shows a squash-holding rack loaded with squash. As designed, this rack holds a minimum of 48 squash, or one and one fifth times as many as would be infested daily in a three-man operation. Forty-four racks would be required to carry out such an operation. Reducing the over-all height to accommodate 7 trays instead of 8 results in a rack capable of holding 42 squash, or two days' output, in a two-man operation. Figure 17 shows the structural details of a rack for 42 squash. Approximately 30 such racks would be required.

Trays for these racks are 42 in. long and 16 in. deep. For a three-man insectary, a total of 352 such trays would be required; for a two-man operation, 210 trays. The metal frame for these is made of $\frac{3}{4} \times \frac{3}{4}$ inch angle iron. One-half inch hardware cloth forms the bottom and a hardwood brace is placed across the middle, as shown in figure 18. This brace lends support to the hardware cloth and minimizes sag.

Fig. 16. A squash-holding rack loaded with scale-infested squash.

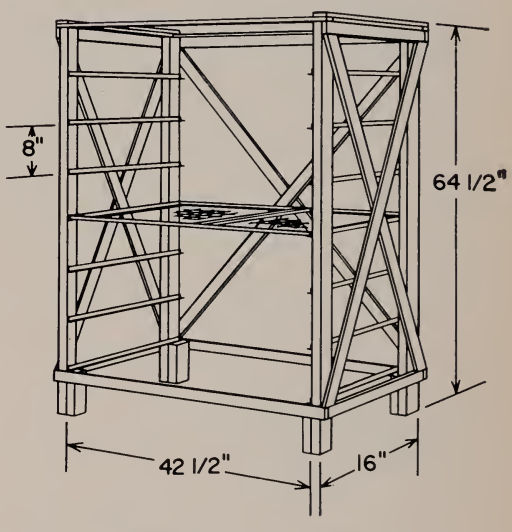


Fig. 17. Details of a squash-holding rack.

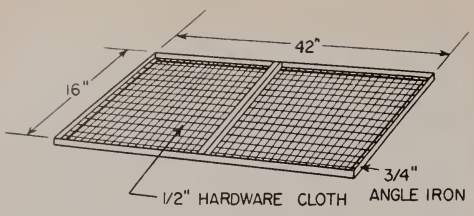


Fig. 18. Details of a squash-holding tray.

Fig. 19. Individual squash holder.

Other Items Required. Approximately 280 squash holders are required for use in the crawler-collection rack (see fig. 1, p. 27) and in the infesting work. These are pictured in figure 19, and are made of door-stop material, the two side rails being made of $\frac{3}{4} \times \frac{1}{4}$ inch door-stop and the two ends of $2 \times \frac{1}{4}$ inch door-stop or molding.

The parasite dispenser shown in figure 20 is made from the lower end of a 6-inch Pyrex test tube. To this, a short section of 8-mm glass tubing is affixed in such a way that parasites can be poured from the reservoir into the stem and then leveled off by rocking the dispenser back to an angle approximating 45° (see fig. 20). This leaves the stem full of parasites. The length of the stem determines the number of parasites dispensed into each carton. The pictured dispenser places 4,000 female *Aphytis* in each field-release carton.

The entire dispensing process may be further speeded by preparing a device incorporating a rocking rack. This rack might hold from 4 to 10 such dispensers, permitting the filling of several field-release cartons rather than one at each operation. A closing device would be required for the tube on each dispenser, and these could be linked to and operated by a single lever. This suggested mechanization might be carired one step further. The strips of honeyed paper for use in field-release cartons might be prepared mechanically on a simple machine capable of rolling the honey out to a thin

Fig. 20. Tipping the parasite dispenser to fill the delivery tube.

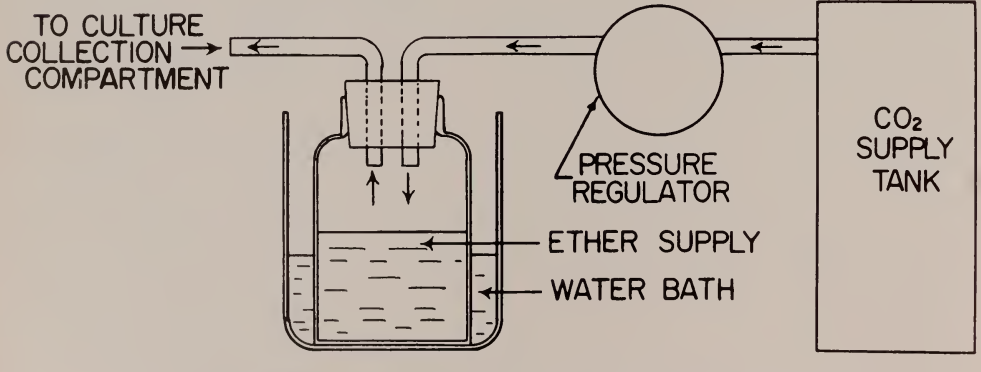


Fig. 21. Diagram of the carbon-dioxide-ether parasite-anesthetization apparatus.

layer and equipped with a row of knives which would cut the honeyed paper into strips of the proper width.

The carbon dioxide and ether dispenser used in this method was developed by Finney, Flanders, and Smith (1947). The ether container is held in a water bath (room temperature) which eliminates frosting and protects against breakage of the ether flask. This system is diagrammed in figure 21.

Finally, refrigerated storage facilities for packaged parasites must be provided. A small commercial reach-in refrigerator serves well with slight modification. A temperature of 60° F must be maintained and few commercial installations utilize a temperature this high. Replacement, or sometimes simple adjustment, of the temperature regulator will usually suffice. Such a cabinet must provide a minimum of 8 sq. ft. of usable shelf space, i.e., shelf space with at least 8 inches of clearance above it.

Equipment costs are discussed in a later section.

INSECTARY PEST CONTROL

One of the omnipresent problems in any insectary operation is the maintenance of pure cultures. Contaminant-free cultures must be maintained in order to insure a continuous flow of host material into the parasite-production rooms.

By its very nature, the present method inhibits the establishment and increase of most insectary pests. In the collection of scale crawlers at a shadow line, most positively phototropic winged insect pests (for example, parasites) are automatically separated from the new infesting material. Such separation is essential to the reëstablishment of a clean culture once the host culture has become contaminated. In the parasite-production phase, the relatively short life cycle of *Aphytis* is advantageous in holding most pest populations in check. The oviposition-collection units serve as isolation units in this case, and these prevent the spread of pest species.

The insectary pests which have been observed in this laboratory could be divided into two groups: (1) the phytophagous pests, represented by certain mites and scale insects, and (2) entomophagous insects including parasites (primarily *Aspidiotiphagus* sp.) and predators [primarily *Lindorus lophantae* (Blaisdell)].

Phytophagous Pests. During the early phases of the present work, phytophagous (plant-feeding) mites were considered a serious pest, primarly because the extent of damage which they could inflict on the squash was unknown. But it has been our experience that mite populations, regardless of the density or magnitude of such populations, do no measurable damage. In fact, dense mite populations occur only on those squash on which the scale do very well. Neither population interferes with the other. The one problem presented by this contaminant involves the webbing left by the mite on the surface of the squash. This webbing must be removed in an operation which will be described later.

Scale-insect contaminants have presented no problems during the course of these investigations, but certain precautions (see "Control by Exclusion of Pest Species," below) have been observed in order to prevent their entry to the scale-rearing area; and difficulties can be foreseen in case such a contaminant becomes established. A hardy contaminant of the diaspine group might become dominant in competition with oleander scale. A lecanine contaminant would interrupt crawler collection with its honeydew secretions. Either hard or soft scales

would seriously disrupt the scale-production program, and thereby increase the cost involved in such a program.

Entomophagous Pests. Among the insect pests that directly attack the scale, parasites comprise the greatest single threat to a scale culture. They are highly motile and are capable of a rapid and continuous build-up on oleander scale. If left unhindered, parasites can destroy a scale culture in a short time.

Predators as a class rank second only to the parasites as a threat to an insectary scale culture. Unless their activity is limited, coccinellids such as *Lindorus* can be quite as destructive as parasites.

Control by Exclusion of Pest Species. Since insect pests generally present a definite threat to the maintenance of the host scale, and therefore to the maintenance of *Aphytis* production, it is very important to exclude such contaminants from the rearing areas. This can be done very easily if simple precautions are observed unwaveringly. These include: (1) Personnel should move about the insectary with caution. The scale-production area should not be entered by anyone engaged previously in the parasite area. Personnel returning from the field (those engaged in parasite-release work) should not enter any portion of the insectary until all chance of carrying a contaminant into the rearing areas has been eliminated. Release of parasites late in the day so that personnel so engaged would not have to return to insectary duties, or a shower and change of clothes for such personnel, may be desirable. (2) All equipment and materials being moved into the insectary should be free from any organism which might pose a problem to the continuous production program.

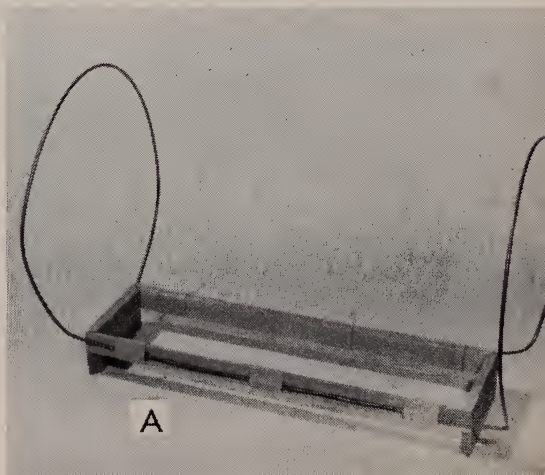
In short then, the best method of pest control in the insectary is total exclusion of such potential pest species.

Control of Established Pest Species. In the event of an outbreak of a pest species in the insectary, no choice remains to the operator but to initiate immediately a program of eradication directed against the contaminant. This is true of all of the above-mentioned pest groups except the phytophagous mites. Eradication is essential once such a pest becomes established but can be difficult and expensive. Several techniques may be used in the eradication of an entomophagous pest. Four of the standard procedures, which are usually used in combination, follow.

1. Isolation of the culture or portions of the culture from the source of contamination is the finest single tool which the insectary man can use. Isolation may vary in degree from a single unit housing the entire scale culture to many small units, each containing a single squash. Isolation is valuable not only in eradicating a troublesome contamination, but also in preventing contamination. A large culture broken into two or three isolated segments reduces the chance of heavy losses due to a devastating pest attack in that large portions of the culture may be spared such attack.

Regardless of the degree, isolation should be adequate to insure that no contaminant will reach the newly infested squash until they are ready for crawler or parasite production. In the normal course of things in an insectary, a room

Fig. 22. A, Squash holder equipped with wire isolation sleeve holder;



is generally an inadequate isolation chamber for use in an eradication program since, on each trip into the room made by the insectary man, the contaminant may enter.

The isolation of each newly infested squash in a chamber holding just one squash is the ultimate degree of isolation. Figure 22 shows an inexpensive unit for the isolation of individual squash. The rack is one of the regular squash holders to which wire hoops have been attached. The sleeve is made of percale. A plastic window is used in order to check progress of the scale, observe the date on the squash surface, and check for contamination. Such a window is not essential to the use of the sleeve if the infesting date is placed outside the sleeve. The check for contamination can be made at the time the sleeve is removed.

2. An equally important technique, and one closely related to isolation, is separation of host and contaminant in the process of infesting host material. This is done automatically during the crawler-collection process, since the scale crawlers stop at the shadow barrier and insect parasites or predators fly to the light source and are eliminated. This technique is of primary importance, for continued reinfestation by the contaminant along with the host would defeat the purpose of an eradication program.

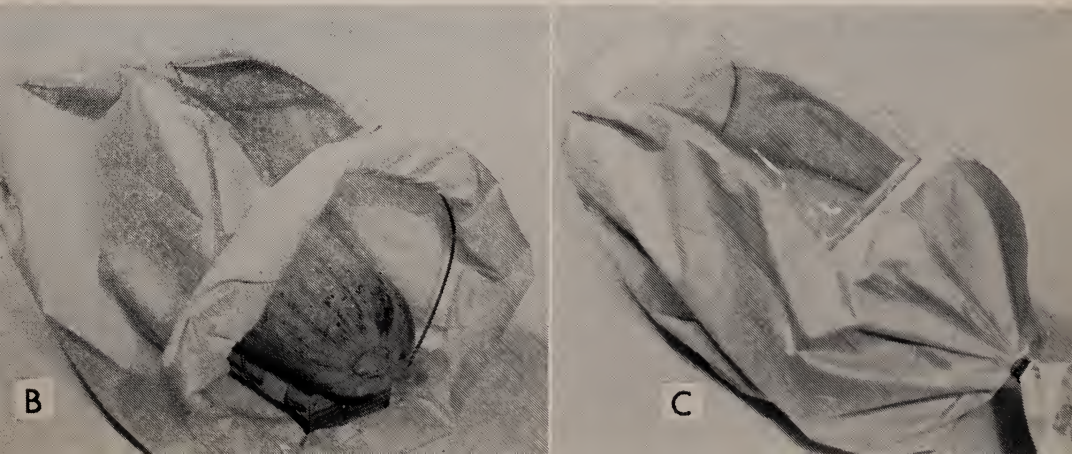
3. Differential insecticides or certain insecticides applied at a differential rate may be used to advantage in some circumstances if necessary precautions are observed for protecting the culture insect.

4. Eradication of the pest from equipment or an insectary room may be done by fumigation with methyl bromide or TEPP. Since fumigation with either of these materials is **extremely dangerous,** it should be attempted only by trained and experienced personnel. Detailed instructions are therefore beyond the scope of this publication.

Use of Insecticides in the Insectary. The utilization of any insecticide in the insectary requires extensive analysis of the problem including the immediate and residual effects of the insecticide on the culture insect, the contaminating insect, and the host plant. Ill-advised or promiscuous use of any insecticide could be dangerous to the production program. On the other hand, careful and considered use of some insecticides can be an extremely valuable aid in the control and eradication of pest species.

In order to make possible the complete separation of crawlers and unwanted parasites at the time of infesting, it is necessary to control the **density** of the contaminating parasites. Such control can be had by utilizing certain insecticides and applying them at a differential dosage. Pyrethrum applied at a differential rate has been very useful in this

B, infested squash in isolation sleeve; C, sleeve closed.



laboratory in the control of a pest-parasite population in the oleander-scale culture during an eradication program.

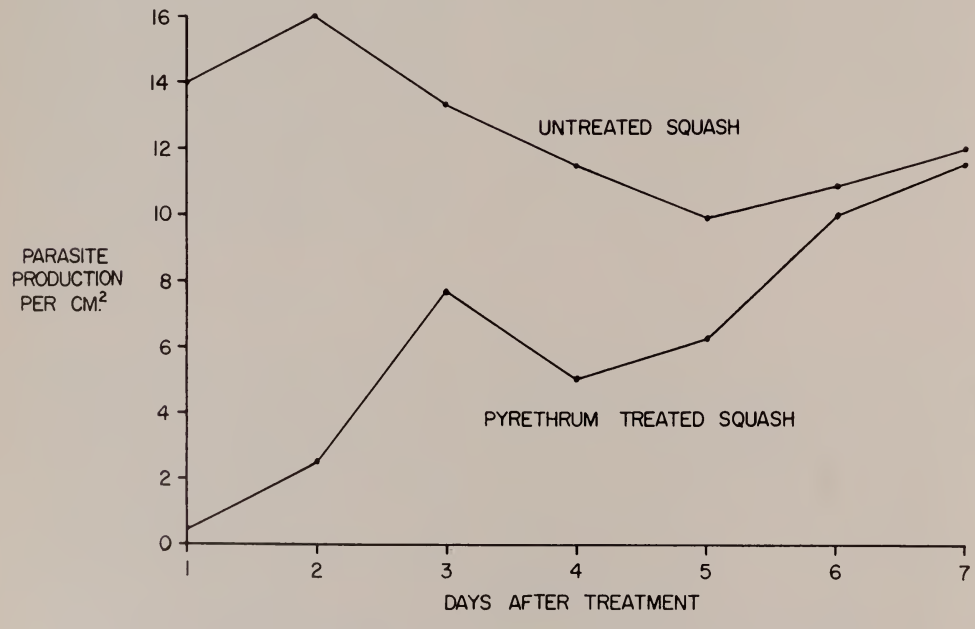
The immediate and residual effects of pyrethrum on oleander scale. *Aspidiotiphagus* sp. (the contaminant), and on the host plant (banana squash) have been investigated. The pyrethrum source was a commercial aerosol bomb which utilized soybean oil as the carrier and freon as the propellant. Dosage was measured in the number of seconds during which the aerosol was permitted to escape into a space of 1,000 cubic feet. Dosages ranging from 2 seconds per 1,000 cu. ft. to 20 seconds per 1,000 cu. ft. were used.

The results of this series of tests indicated that a dosage of 6 seconds per 1,000 cu. ft. did maximum damage to the pest-parasite population and minimum damage to the scale. This treatment showed no measurable effect on the surface of the squash. Scale crawlers and newly settled scale were susceptible to the pyrethrum treatment. However, after the scale reach 14 days of age or older, treatment at this dosage produces no ill effects.

The adults of the pest parasite present at the time of treatment were completely susceptible to the aerosol, and the residue on the upper surfaces of horizontal planes is adequate to kill them for periods of at least 24 hours. These factors indicate the excellent potential which this insecticide may have in the control of a pest-parasite population during an eradication program.

The effects of pyrethrum treatment applied in the scale-production area upon later oviposition by *Aphytis lingnanensis* in the parasite-production area were investigated. Oviposition by *Aphytis* on untreated squash was compared to that on treated squash where the residue ranged in age from 1 day to 10 days from the last day of treatment. Results are shown in graph 7. A marked difference in production of parasite progeny is indicated until after the fifth day of aging or dissipation of the residue, this becoming negligible on the sixth day.

This would indicate that, unless steps were taken to remove treatment residue, treated squash being transferred to the



Graph 7. Effect of pyrethrum aerosol residue on parasite-progeny production.

parasite-production area should be held for five days prior to sting without further treatment in order to permit necessary aging or dissipation of treatment residue.

Other insecticides have been useful in the insectary. Fisher and Dawson[9] used a 5 per cent chlordane dust sprinkled on the floor in a red-scale culture room which had become contaminated with the potato tuber moth, *Gnorimoschema operculella* (Zell.). Larvae of the potato tuber moth in preparing for pupation dropped to the floor and were destroyed.

In combating *Lindorus lophantae*, lead arsenate was applied as a dust to the surface of the scale-infested squash. *Lindorus* larvae and adults feeding on the dusted oleander scale were rapidly reduced in number. Lead arsenate made it possible to control and eventually eradicate this troublesome pest. The scale stock was not adversely affected.

In the control of phytophagous mites, the commercial products Aramite and Dimite were applied to the squash as a dip prior to infesting with scale or as sprays after infesting. Both preparations aided in the successful control of these mites.

Some Specific Recommendations. The following recommendations are designed to cover specific problems as they may arise in the host-scale culture in the light of the preceding information.

1. Mites: No control is recommended for phytophagous mites because no measurable damage results from a population build-up by such organisms. One precaution must be observed, however. Since the abundant webbing which covers the surface of a squash during and after a mite population build-up interferes with the normal collection of crawlers in the crawler-collection unit and with ovipositional activity of parasites in the sting-collection unit, it must be removed. Removal is accomplished easily by brushing the squash surface lightly with a soft counter brush.

2. Scale insects: A new and contaminant-free host culture must be established and isolated in such a manner as to preclude all possibility of recontamination.

3. Parasites: In the event of contamination of the scale culture by parasites, three steps are advisable: (a) During the infesting process, the collected crawlers must be carefully inspected and any adult parasites removed in order to prevent them from reaching newly infested material. (b) All newly infested material must be isolated in such a manner as to preclude recontamination by the parasites. This isolation must be for a minimum period of 14 days, longer periods being more advantageous and reaching the ideal when protection is afforded until scale are mature. (c) Pyrethrum or some other carefully selected insecticide may be used to reduce the parasite population in the infesting room and in the holding rooms if less than full-time isolation is employed. If newly infested squash cannot be individually isolated for more than 14 days, a program for rotating the protective sleeves may be devised, in which case a daily pyrethrum treatment of 6 seconds per 1,000 cu. ft. will maintain the parasite population at a low level in the holding rooms. Care should be exercised to insure that the youngest scale in the room are not less than 14 days old when the room is subjected to treatment. In connection with this daily treatment program, it would be advisable to rotate all squash 180° each day just before treatment in order to better distribute the treatment residue.

In the crawler-production area, the parasite population can be maintained at a low level by treating the room daily with pyrethrum aerosol. Treatment would follow the day's infesting and requires two preliminary steps: (a) The crawler-collection cards have to be inverted or stored in some protected place in order

[9] Fisher, T. W., and Louis H. Dawson. Unpublished notes.

to prevent the accumulation of treatment residue. (b) The squash in the crawler-collection rack should be pushed back into the rack in order to protect them (and the crawlers emerging on their surfaces) from the treatment residue. Apparently, by restricting the air space above the squash the amount of residue settling on the exposed upper surface of the squash can be reduced. In the scale-production area, also, treatment dosage is limited to 6 seconds per 1,000 cu. ft.

4. Predators: The steps outlined in the control of parasites apply also in the control of predators except that a stomach poison such as lead arsenate is used in place of pyrethrum. Pyrethrum used in dosages large enough to destroy the predator also destroys the host scale.

No specific control measures have been found necessary in the parasite-production work. Since the oviposition-collection units serve as isolation units and *Aphytis* has a short life cycle, no known contaminant is capable of extensive damage during the short period involved in this phase of the work.

One precaution must be observed, however. If any insecticide is applied to the squash during the scale-production period, such squash must be washed in order to protect the inoculation parasites. A gentle stream of water passed over the surface of pyrethrum-treated squash removes or dissipates the residue. When lead arsenate has been applied, the squash must be washed before crawler production begins. Lead arsenate is not so readily removed with a gentle stream of water as is the residue of pyrethrum. However, washing removes some of the lead arsenate dust and wets the balance, which renders it harmless to scale crawlers and to *Aphytis*.

THE INSECTARY

Spatial Requirements. The spatial requirements for an insectary operation of the type and scope presented herein are treated in some detail in the following discussion, since the efficiency of any program is dependent upon the efficient utilization of floor space. The operation of a two-man insectary capable of producing 176,000,000 female parasites during the 9-month release period will form the basis for this discussion. Such an operation could serve 441 acres or more at a cost substantially below the $40 per acre cost figure mentioned earlier.

The room which houses the crawler-collection rack must be at least 20×20 feet, and if one of these dimensions were greater, infesting of squash would be expedited, in that this additional space would permit infesting squash in the room in which crawler collections are made. In the insectary plan shown in figure 23, the crawler-collecting and squash-infesting room is 20×28 feet, providing adequate space for squash handling and infesting. This plan is designed to accommodate the two-man operation. An insectary designed for a three-man operation could be built from a similar plan in which the squash-holding and parasite-culture space would be expanded by approximately one third.

Holding space (the area in which the squash are held during the scales' growth and maturation period) must be large enough to hold 28 racks of squash, allowing 20 inches in width and 44 inches in length for each rack, plus reasonable access ways. Figure 23 shows two rooms, each 7×24 feet, utilized as holding rooms, and this represents the minimum number which should be used. Additional holding rooms which permit the isolation of still smaller segments of the scale

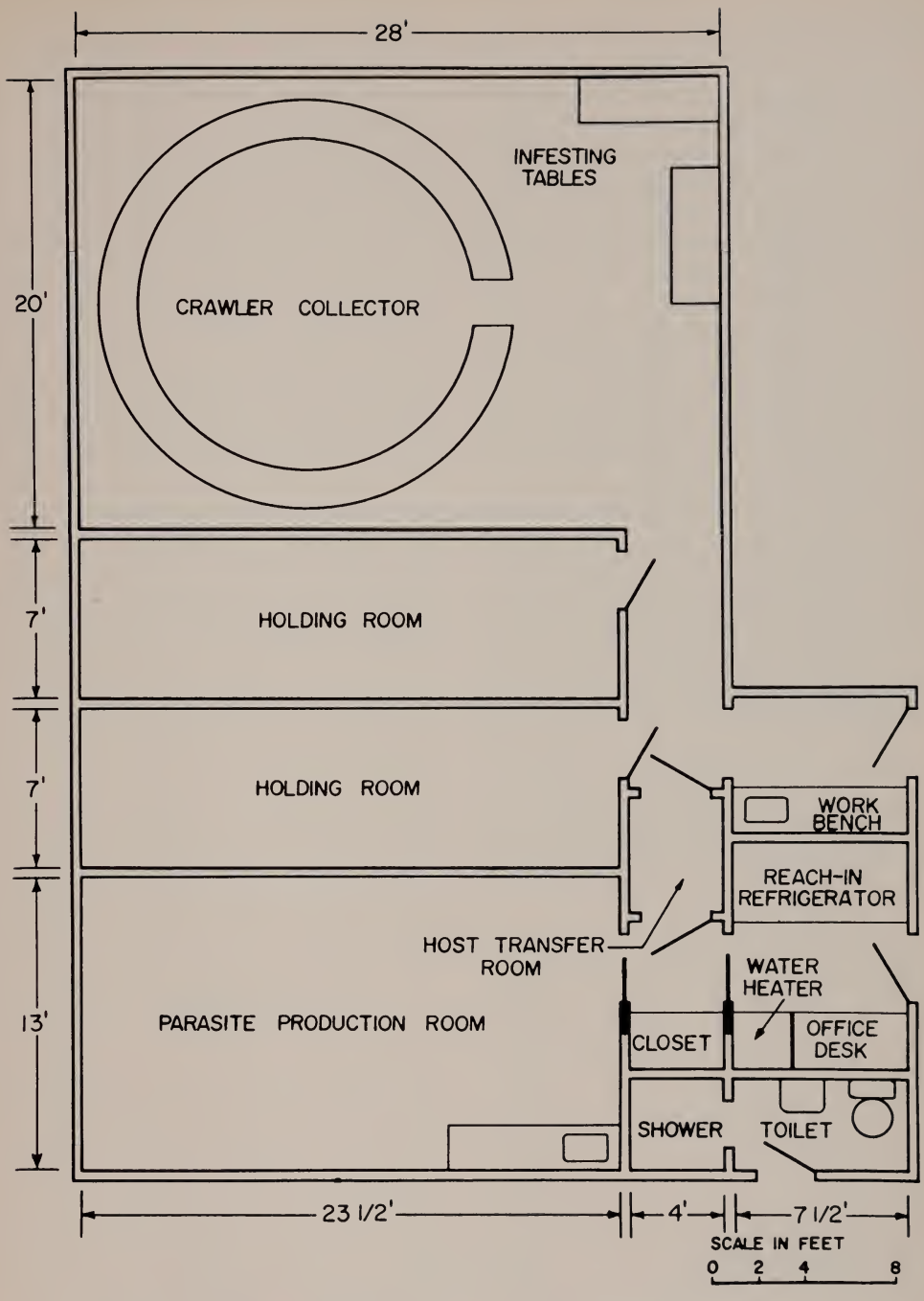


Fig. 23. Floor plan for a two-man Aphytis production insectary.

culture would be invaluable in cases of pest insect outbreaks.

Since the squash used in the parasite culture are isolated in the compartments of the sting-collection units, a single room for the entire parasite culture is desirable but not essential. The room shown in the plan measures $13 \times 23\frac{1}{2}$ feet and is more than adequate for the entire parasite culture.

The complete absence of windows makes this insectary plan unique. Windowless construction possesses two distinct advantages: (1) control of temperature and humidity are facilitated, and (2) the intensity of artificial light illumination in the scale-infesting and parasite-production areas can be controlled easier than can natural sunlight.

Since it is necessary to control temperature and humidity in the production areas, insulation of the building should prove to be a wise economy. If the building is constructed on a concrete slab with walls of cinder-block or concrete brick, insulation of only the roof (or attic) should be adequate.

It will be noted that the transfer cubicle just off the entrance hallway of the parasite-production area is utilized as the receiving point for host material. This small room should be black-lined and should contain a continuously operating light-trap. Scale-infested squash would be placed in this cubicle early in the day and then removed immediately into the parasite room. Such an arrangement would permit maximum time for the trapping of any parasite which found its way into this cubicle from the immediately adjacent parasite room. This arrangement is probably as safe as any practical method for transfer of host material.

In the plan shown in figure 23, office space, toilet facilities, cooled parasite-storage space, and additional working areas are provided. This plan is straightforward and simple. Construction costs are held to a minimum and when depreciated over a ten-year period, should not

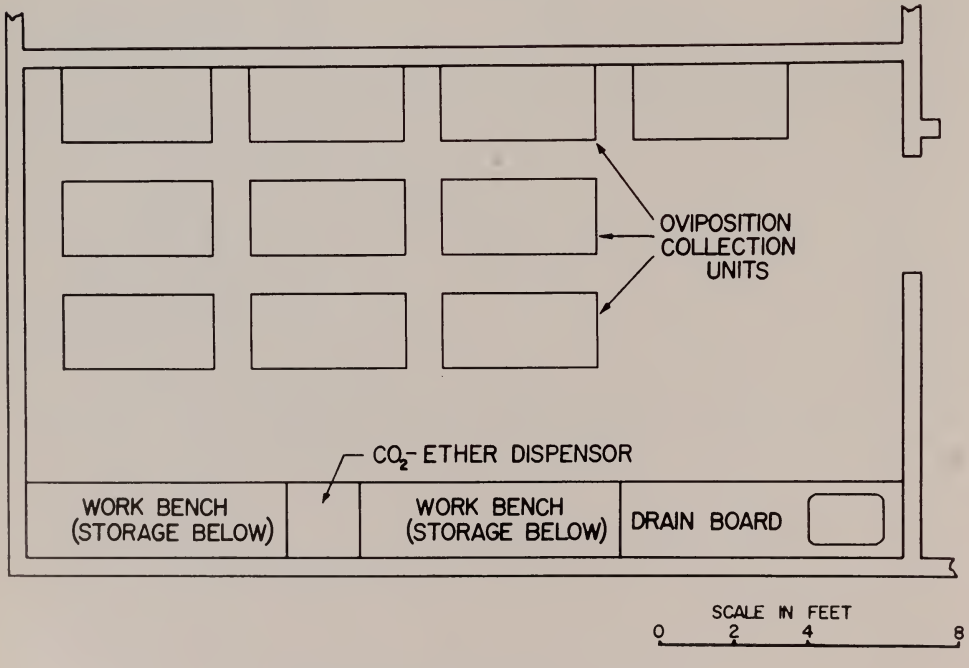


Fig. 24. Parasite-production room layout.

exceed the figure given as a housing estimate in table 11.

Typical layout for the parasite-production area is shown in figure 24.

Climate Control Equipment. As indicated earlier, the entire rearing area for both scale and parasites should be air-conditioned. The equipment should be capable of maintaining a temperature of 75° F with a relative humidity of from 50 to 55 per cent in the scale-production area, and a temperature of 80° F with relative humidity near 50 per cent in the parasite-production area. Separate auxiliary fans in each room should be used to prevent stratification of air in layers of differing temperatures.

In the entire rearing area, a 7-foot ceiling is desirable because temperature and humidity control is easier with a low ceiling and layering of the air in temperature belts is minimized.

Entrances. Separate entrance areas should be provided for each rearing area. In this manner, the parasite and host areas are isolated to a greater degree, and the possibility of accidental transfer of the parasite to the scale culture is minimized. The floor in these entrance areas should be level with those in the rearing areas and connected by ramp to the ground level or the floor level in the squash-storage area.

Conversion of Existing Structures. In evaluating the possibilities of converting an existing structure into a useful insectary for the purposes presently under consideration, the spatial requirements enumerated above must be borne in mind. The absolute isolation of scale and parasite cultures must be observed.

The conversion of an otherwise acceptable building usually involves some modification in the electrical wiring. Though the operation of heavy electrical equipment other than that used for air conditioning is unnecessary, circuits must be adequate for ample working light, and should feature convenience for the insectary man.

Doors and windows should be made insect-tight. All outside entrances not used should be sealed.

Finally, as recommended for new structures, the insectary should be insulated in view of the savings afforded in control of temperature and humidity.

Squash Storage. A suitable structure is required for squash storage outside the insectary. This structure must be large enough to accommodate 25 tons (for a two-man insectary) of squash and should be rodentproof. A structure screened with ½-inch hardware cloth and covered with a sheet-metal roof is adequate. Minimum area so enclosed should be about 525 square feet with a ceiling not lower than 8½ feet. Figure 25 shows the floor plan and rack arrangement in a typical squash-storage area. The structure represented by this plan is 23½ × 23½ feet.

All racks are ruggedly built of dimension lumber in order to support a heavy squash load and have shelves spaced at 18-inch intervals from floor to ceiling.

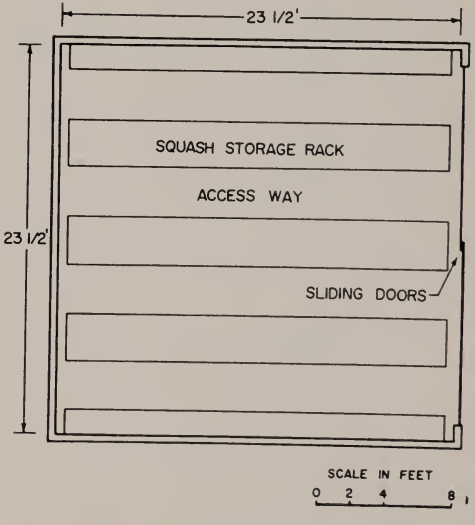


Fig. 25. Squash-storage layout.

The storage racks against the outside walls should be 18 inches deep, the rest 34 inches deep and accessible on both sides. Squash are loaded into these racks from each side and placed three rows deep on each shelf. As indicated, three racks, each 34 inches wide, and two racks 18 inches are required.

ECONOMICS OF THE PRODUCTION METHOD

It has been pointed out that the present work was undertaken in an effort to develop a method of *Aphytis lingnanensis* production which is reliable and economically feasible to compete with spray treatments for the control of California red scale, which cost approximately $40 per acre per year. An economic program then must include not only the rearing but also the colonization of parasites.

The method of production herein described can be used in a variety of ways and is in no way limited to use in an insectary of any given size or production capacity. In order for production and release of parasites to proceed on a cost basis lower than insecticidal treatment, however, operation must be maintained at or above a minimum level.

The possibilities of successful and economical production by one-man, two-man, and three-man production units have been evaluated. An insectary operated by one man can produce enough parasites to service a minimum of 285 acres, but one man would not have sufficient time to liberate the parasites produced. Cost of production alone in this case would approximate $37 per acre served.

At least 441 acres could be served by a two-man production unit, and in this case all field liberations are made by the production personnel. Production and colonization costs combined are estimated at $38 per acre. Therefore, two men operating the insectary on a 7-day basis during the 40-week release schedule form a desirable minimum production unit which can be maintained at a cost figure below the $40 per acre figure referred to earlier.

Each man in such an operation would be expected to work 44 hours per week during the 40-week release period, with each man taking vacation time during the fall or winter months when the work load is substantially reduced. A complete work week under peak work load has been outlined in table 10. The work expected of the two men has been divided into three portions: that dealing with (1) scale-culture maintenance, (2) parasite production and packaging, and (3) liberation of parasites produced. Scale production should require not more than 2½ hours daily, while parasite-production work should require 5½ hours or less. Liberation work (colonization) requires a minimum of about 3½ hours daily, and on two occasions each week the entire day's production will have to be stored overnight and colonized on the following day. Under peak work conditions, 9 hours remain unassigned for each work week. This time can be spent in general insectary maintenance.

In tables 11 and 12, estimates of production costs based on a two-man insectary operation are itemized, parasite production is computed and the cost per acre served is indicated. Necessarily, most of the items in table 11 are calculated estimates and are subject to variation from locality to locality, most obvious of these, of course, being the cost

Table 10. Typical weekly work schedule in a two-man *Aphytis*-production insectary

Time of day	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Activities of man no. 1							
8 a.m.–12 m.	Recovery	Recovery	Recovery	Recovery	Recovery	Recovery	
	Packaging	Packaging	Packaging	Collecting	Packaging	Packaging	
				Sting			
	Unassigned	Unassigned	Unassigned	Unassigned	Unassigned	Unassigned	
1–5 p.m.	Infesting and rotating squash	Infesting and rotating squash	Infesting and rotating squash	Infesting and rotating squash	Infesting and rotating squash		
	Colonizing	Colonizing	Colonizing	Packaging	Colonizing		
Activities of man no. 2							
8 a.m.–12 m.							Recovery
	Collecting	Collecting	Collecting		Collecting	Collecting	Collecting
	Sting	Sting	Sting		Sting	Sting	Sting
	Colonizing	Colonizing	Unassigned		Colonizing	Unassigned	Unassigned
1–5 p.m.	Colonizing	Colonizing			Colonizing	Infesting and rotating squash	Infesting and rotating squash
						Colonizing	Packaging

Table 11. Annual production costs in a two-man Aphytis-production insectary

Based on costs at Riverside, California, in 1956

Item	Total estimated cost	Cost per year
Equipment:		
Sting collection units (10 @ $350)	$3,500	$ 700*
Squash racks (30 @ $50)	1,500	300*
Squash rack trays (225 @ $3)	675	135*
Squash holders (180 @ $0.50)	90	18*
Crawler collection rack	150	30*
Cooled storage chamber	300	60*
Supplies:		
Host plant—40 tons	...	1,680
CO_2	...	450
Ether	...	500
Miscellaneous	...	800
Salaries	...	8,000
Interest on equipment	...	373
Housing	...	2,100†
Utilities	...	600
Transportation	...	1,200
Total cost per year	...	$16,946

* Annual depreciation cost for equipment calculated on the basis of a five-year life.

† Annual depreciation cost for building calculated on the basis of a ten-year life.

Operational Estimates (Do Not Include Insurance and Taxes)

	Two-man insectary	*Three-man insectary*
Annual squash tonnage	40 tons	64 tons
Peak squash storage	25 tons	40 tons
Squash storage area	525 sq. ft.	840 sq. ft.
Squash infested daily	21	34
Squash in mother culture	126	204
Squash stung daily	18	30
Annual production	176,400,000 females	294,000,000 females
Parasite cost per thousand (including colonization)	$ 0.096	$ 0.081
Parasite cost per acre (including colonization)	$ 38.30	$ 32.40
Total production cost per year (including colonization)	$16,946.00	$23,836.00
Total acres served	441	735

of housing, which varies sharply with location, existing facilities, and so on. All estimates were based on the costs of these items in the Riverside area of southern California during 1956.

It should be noted that this appraisal of the economics involved in the parasite-production program is predicated on the proposition that all of the 441 acres being served require the maximum colonization recommended, 400,000 females per acre. If any substantial portion of this acreage is in a more favorable climatic location (coastal or near coastal), cost of parasite production per acre could be reduced and larger acreages could be served.

As indicated earlier, an enlarged operation would reduce costs substantially. For example, a three-man production-unit could produce enough parasites to serve over 700 acres at a cost approximating $32 per acre. In the tabulation on page 54 certain features of a two-man insectary are compared with those of three-man operation.

Table 12

Amount of parasite production and cost per acre for a two-man Aphytis production insectary

1. Daily production
18 squash daily × 35,000 females per squash = 630,000 females produced daily
2. Annual production
Item 1 : 630,000 × 280 production days = 176,400,000 females produced annually
3. Cost per thousand
$16,934 ÷ 176,400 = $0.096 per thousand
4. Cost per acre
Item 3 : $0.096 × 400,000 female parasites per acre per year = $38.30
5. Acres served
Item 2 : 176,400,000 ÷ 400,000 = 441 acres

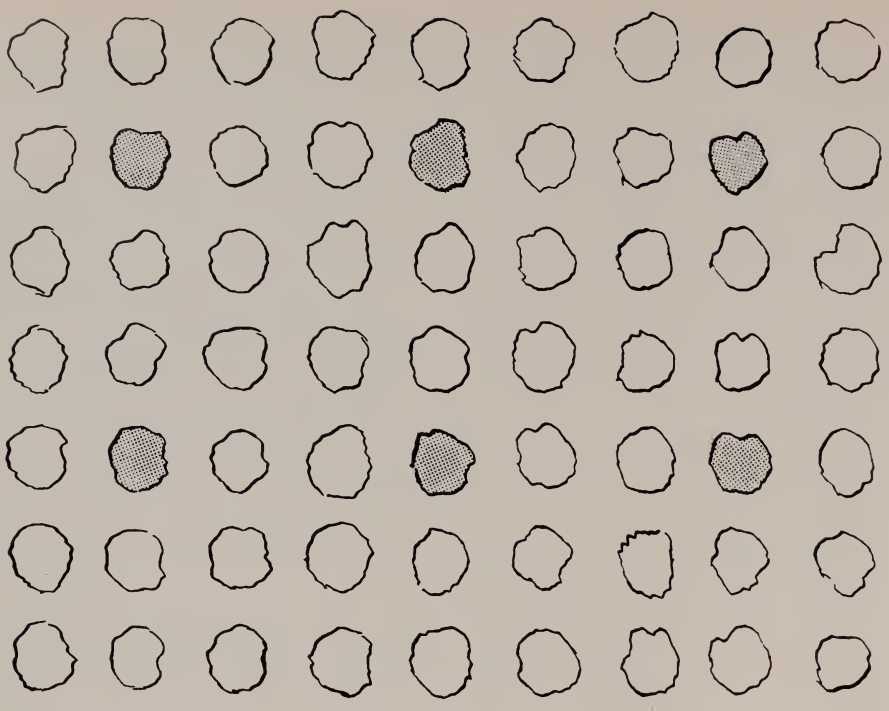
FIELD COLONIZATION

The ultimate goal of mass production of parasites is colonization in the field. Colonization is considered an integral part of the whole method from the standpoint of figuring cost. In our method, the men producing the parasites also handle the field colonization. The transportation expenses incurred are figured into total operating costs.

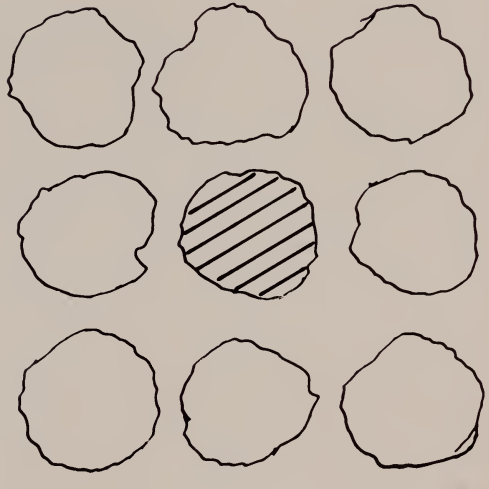
Field colonization has to be "streamlined" or costs will be excessive. For instance, the method used in colonization of test plots or for colonization of newly imported parasites is commercially impractical. It involves the use of expensive tubes, with removable plugs in each end, which contain 1,000 parasites each. Large numbers cannot be used per tube because of adverse effects from crowding. To colonize parasites, the plugs are removed and the parasites are blown onto the tree with a sharp puff of breath. This is time-consuming, for each tree must be visited and, in addition, a fairly large bulk of tubes must be handled. A more rapid and economical method was therefore devised.

Method. It will be recalled that the objective was to colonize 400,000 female *Aphytis lingnanensis* per acre per year. Field comparisons showed that results from 9 equal monthly colonizations (March through November) were equal to or better than colonization at any other combination of intervals. This means (on the basis of 100 trees per acre) a total of 4,000 parasites per tree per year. A simple and economical colonization method to meet this requirement follows.

First, it is necessary to visualize a grove as being composed of groups of 9-



tree blocks. A 9-tree block, as shown at the right, can be considered to consist of a center tree with 8 trees grouped around it in a square. Parasites colonized in the center tree can disperse readily to the immediately adjacent trees; hence, the center tree alone can serve as a colonization focus for the 9-tree block. If 4,000 female parasites are colonized monthly on the center tree of a 9-tree block from March through November, then the average per tree per year will be the desired 4,000 female parasites, or approximately 400,000 per acre per year. To carry out this program it is merely necessary to map the grove into 9-tree blocks, although in a square or otherwise uniform grove this may not even be necessary, as a glance at the schematic representation (above) of a small grove will show.



In no case is any given tree more than one tree removed from a colonization tree.

Since this scheme calls for colonization of 4,000 females on a single tree at any given time, it is best to package parasites in units of 4,000. Half-pint waterproof cardboard cartons were found to be ideal for this purpose. There is ample "roosting" space for 5,000 parasites (4,000 females) in one of these cartons,

and the colonization is simply accomplished by removing the top and placing the carton in the crotch of the desired tree. Parasites will rapidly leave the carton, crawl up the limbs, disperse throughout the tree and ultimately fly to adjacent trees.

Since only 10 or 11 trees are colonized per acre and about 15 acres are colonized daily during the season, one insectary man under a minimum-size commercial insectary program should be able to do this work in about 3½ hours a day.

Colonization should be carried out under weather conditions as favorable as possible for the parasites. Releases should not be made just before or during rains, on days not likely to exceed 60° F, during heavy winds, or late in the day if frost may occur at night. In the latter case, it is best to colonize in the morning, so that the parasites have a chance to move about during the day and find places of shelter if such are necessary.

When transporting parasites to the field care should be taken that they are not subjected to excessive heat or cold. During hot periods (field temperatures over 90° F) parasite containers should be covered with a moist wrapping or carried in an ice chest during transportation. Temperatures in the part of the ice chest containing parasites should not be below 55° F if parasites are to be held for any length of time.

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